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REPORTS

TO THE

LOCAL GOVERNMENT BOARD

ON

PUBLIC HEALTH AND MEDICAL SUBJECTS.

(NEW SERIES No. 40.)

Further Reports (No. 3) on Flies as Carriers of Infection:—

1. Observations on the ways in which artificially infected flies (*Musca domestica*) carry and distribute pathogenic and other bacteria: by Dr. Graham-Smith.
2. Summary of literature relating to the bionomics of the parasitic fungus of flies (*Empusa muscæ*): by Dr. Bernstein.
3. Note as to work in hand but not yet published, and as to proposed further work in reference to Flies as Carriers of Infection: by Dr. Copeman.

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TO THE RIGHT HONOURABLE JOHN BURNS, M.P., PRESIDENT OF THE LOCAL GOVERNMENT BOARD.

SIR,

I SUBMIT herewith a third report in continuation of the investigation authorised by you on Flies as Carriers of Infection. The contents of the two reports already published are given on the first page of the present report.

Dr. Graham Smith's elaborate experiments on flies, described in the present report, throw important new light on the means by which flies may serve to carry infective material and communicate it to food. A summary of these experiments is given on page 39. They show that non-spore-bearing bacteria frequently survive for several days in the crops of flies; and that, after a meal, flies may regurgitate or "vomit" some of the contents of their crops through the proboscis. These facts are suggestive, but Dr. Graham-Smith is cautious in drawing wide inferences from them pending further experiments.

Dr. Bernstein discusses on page 41 the possibility of aiding the spread of the fungus disease to which the housefly is subject; and Dr. Copeman on page 45 summarises the further work of investigation on flies now in hand.

I am, Sir,

Your obedient Servant,

ARTHUR NEWSHOLME.

August 10*th*, 1910.

Third Report on Flies as Carriers of Infection.*

Observations on the ways in which Artificially-infected Flies (*Musca domestica*) carry and distribute Pathogenic and other Bacteria. By G. S. Graham-Smith, M.D. University Lecturer in Hygiene, Cambridge. (VII. Plates. 25 Tables.)

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* (1st Report issued June, 1909, dealt with the following subjects :—
1. How to Distinguish the more Important Species of Flies found in Houses.
2. Mr. E. E. Austen's Notes on Flies examined during 1908.
3. Mr. Jepson's Report on the Breeding of the Common House Fly during the Winter Months.)

* (2nd Report issued October, 1909, dealt with the following subjects :
1. Memorandum on Lines of Investigation : by Dr. Copeman.
2. Notes on Colouring Flies for Purposes of Identification : by Mr. Jepson.
3. Preliminary Note on Examinations of Flies for the Presence of *Bacillus Coli* : by Dr. Graham-Smith.
4. Abstracts of Literature and Bibliography : by Professor Nuttall and Mr. Jepson.

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Introduction.

Last year (1909) Nuttall and Jepson contributed to these Reports a very complete summary of the experiments and observations of previous investigators relating to the spread of infectious diseases by *Musca domestica* and allied non-biting flies. *Inter alia* these records show that hitherto, comparatively little attention has been paid to systematic investigation of the subject from the experimental point of view.

The experiments recorded in this paper, carried out during the latter part of 1909, were undertaken to ascertain during what periods of time certain micro-organisms could be recovered in culture from the legs and wings the crop and intestinal contents and faeces and other deposits of flies artificially infected by feeding on cultures. Some observations were also made on the infection of food materials by flies and on the infection of previously uninfected flies, by means of the deposits of infected flies.

Musca domestica was the only species with which experiments were made.

The virulence of the micro-organism used was not determined in most cases, either before or after the experiments, but the identity of the cultures isolated from the flies with those used for experimental purposes was proved by subcultures on suitable media.

During the progress of the experiments many new points of interest from time to time appeared. Some of these problems were dealt with as opportunity permitted, but others had to be left very incompletely investigated although the experiments were carried on till the middle of December by which time flies were only obtained with difficulty.

In describing these experiments it has been thought best to arrange them, not in the order in which they were done, but in groups bearing on the same subject.* In order that the reader should have before him an account of the experimental work which has hitherto been done on each subject the records cited by Nuttall and Jepson of experiments relating to each micro-organism employed have been referred to in the sections dealing with them.

A.—Methods.

Flies were captured in balloon traps baited with sugar moistened with stale beer or treacle and kept until required in a large gauze bag, about 12 inches in diameter and two and a half feet in length, suspended by a string. The sides of the bag were supported by wire hoops and the bottom was composed of a wooden disc. In the latter a square hole was cut the sides of which were fitted with grooves so that in place of the cardboard panel which usually filled the space, a tray, containing watch-glasses of syrup and water, could be inserted, in order to supply the flies with food. A sleeve sufficiently large to admit the arm and communicating with the interior of the bag was attached about half way up. When it was not in use the sleeve was closed by a string tied round it near its junction with the bag. (Plate I., Fig. 3.) The flies, when required, were captured by means of a large test-tube about 12 inches long, which was inserted through the sleeve. The mouth was placed over flies as they walked on the inside of the bag. Once in the tube the flies fell to

* The arrangement of Subjects is indicated in the Summary of contents.

the bottom and occasional shaking prevented them from getting out. In this way a considerable number of flies could be caught in a few minutes. After a sufficient number of flies had been captured they were placed in one of the experimental cages. These consisted of cylindrical glass chimneys about three inches in diameter and nine inches in length. One end of the chimney was closed by gauze kept in place by a piece of thin paper gummed round the chimney over the gauze. The other end was open and when in use rested on a clean quarter-plate negative glass. (Plate I., Fig. 1.) Other cages of the same kind but smaller (one and a half by six inches) were also made use of. The transference of the flies from one such cage to another can be very easily accomplished. The fresh cage is placed on the bench with its open end upwards and the full cage with the negative glass still in place is placed on top of it. The negative glass is then slowly withdrawn leaving the two cages in free communication. By taking up the two cages in this position and holding the fresh one in the direction of the light most of the flies can be induced to pass into it. If any difficulty occurs they can be blown from the old cage into the other. A fresh glass plate is then inserted between the cages.

Flies have been kept alive in such cages, with daily transfers to fresh cages, for more than three weeks. It was very rare for a fly to escape or to be injured during the process of transference from cage to cage.

The flies were usually fed once daily. The liquid food (syrup, milk, sputum, &c.) was deposited in separate drops on a clean negative glass, which was placed in contact with the one on which the cage stood. The cage was then slightly tilted and slipped into position over the food on the new glass. Infected food was given in the same way.

In order to obtain a few flies from a cage for cultural purposes the following plan was adopted. A piece of wood about six inches square, in which a round hole, slightly larger than the diameter of a cage, had been cut, was lined with cloth so as to closely grip the sides of the cage when the latter was placed in the hole. To one edge of the cloth gauze was sewn to form a conical bag about six inches in length and about two inches in diameter at its free end which was open (Plate I. Fig. 2.). When in use the cage is slipped from the negative glass over the wooden frame, and is made to fit into the hole in it. A long test tube is then inserted into the cage through the gauze bag and the required number of flies caught in it. This apparatus worked extremely well for no flies escaped or were injured, in a large number of experiments, during the manipulations.

B.—Anatomy.

Note on certain points of importance in the anatomy of the alimentary canal of the fly.

The œsophagus passes from the proboscis through the cephalic ganglion and neck into the thorax. At the junction of the anterior and middle thirds of the thorax it divides. One part, the crop duct, is continued backwards into the anterior part of the abdomen and becomes expanded into the crop, and the other passes into the proventriculus which is situated immediately above the bifurcation.

The crop is a bilobed sac, capable of considerable distension, which when greatly distended loses its bilobed shape and occupies a large portion of the antero-ventral region of the abdomen. Its walls exhibit unstriped muscle fibres. The proventriculus, into which one branch of the œsophagus passes, is a curious circular organ, flattened dorso-ventrally, and is described by Gordon Hewitt (1907, p. 421) in the following way :—" In the middle of the ventral side it opens into the œsophagus, and on the dorsal side the outer wall is continued as the wall of the ventriculus. The interior is almost filled up by a thick circular plug of cells which have a fibrillar structure, and it is pierced through the centre by the œsophagus. The neck of the plug is surrounded by a ring of elongate cells, external to which the wall of the proventriculus begins, and, enclosing the plug at the sides and above, it merges into the wall of the ventriculus." Beyond the proventriculus is the ventriculus or chyle stomach, followed by the proximal and distal intestine and rectum.

Plate II. fig. 4. is a schematic median longitudinal section of a fly reconstructed from the study of serial sections, showing the relationship of the œsophagus, proventriculus, ventriculus and crop to one another. Plate III. figs. 5, 6 and 7 are reproductions of photographs of longitudinal sections of flies showing these structures.

The structure and function of the crop and proventriculus are matters of considerable interest in considering the distribution of infectious material by flies. At the commencement of a meal, as will be shown presently, the crop is first distended with liquid food. If the feeding is continued after the crop is fully distended, the food may pass directly into the ventriculus through the proventriculus. If, on the other hand, the fly is disturbed before any portion of the food has entered the intestine, the food which has been sucked into the crop is gradually passed into the ventriculus. In any case, after a variable period of time, the contents of the crop pass into the intestine. The proventriculus seems capable, therefore, of being closed during the early part of a meal in order that the food may not enter the intestine but pass into the crop. On the complete distension of the crop, it opens in order to allow food to pass directly from the proboscis to the intestine. It also opens when it is necessary to allow material to pass from the crop into the intestine. After a meal flies usually regurgitate some of the fluid contents of their crops through the proboscis, and, no doubt, during this process the lumen of the proventriculus is closed in order to prevent the fluid from passing into the intestine. Lowne (p. 409) regards the proventriculus as a " gizzard and nothing more," and Gordon Hewitt (1907, p. 421) states that " its structure suggests a pumping function and also that of a valve," while Giles (1906) says that " taking the structure as a whole, it is difficult to resist the idea that it must, in some way, have a valvular function, though it is difficult to say how." The observations just quoted, of which some particulars are given later, seems to indicate that it acts as a valve, possibly controlled, at will, by the fly.

Plate IV., Fig. 8, shows a dissection of the proboscis, œsophagus, crop, proventriculus, and ventriculus of a fly which had been fed on milk and then starved for some time. The crop is nearly empty and the division of the œsophagus is well shown. Plate IV., Fig. 9, illustrates a dissection of the same structures in a fly which had

been fed just previously with liquid gelatin. The distension of the crop is well shown, though much greater degrees of distension have been often met with. In these photographs the organs have been arranged so as to show the several structures to their best advantage and are not in their natural relation to one another. The complete isolation of the structures as shown on Plate IV. is a somewhat difficult dissection, since the œsophagus is very delicate and intimately attached to the chitin in the neck region. It was found, however, that if flies were kept in a cage in a warm incubator (37° C.) they soon gorged themselves on drops of liquid gelatin placed on the floor. On being removed from the incubator after 30–60 minutes, the whole intestinal canal, including the crop, was distended with gelatin and could be dissected out with ease, especially if coloured gelatin was used in feeding. If it was intended to subsequently cut sections, the flies were fixed with formalin.

On several occasions flies which have been allowed to feed on syrup were killed and dissected. The fluid contained in the crop was collected in a capillary pipette and its volume measured. These experiments showed that the capacity of the crop varied between ·003 and ·002 c.c.

C.—Feeding Experiments.

(1.) *Fluids.—Plain and coloured syrups.*

A series of feeding experiments with plain and coloured syrups were conducted in the following manner. Flies which had been kept for 24 hours or more without food were placed in clean cages and a few drops of syrup, made by dissolving brown sugar in water, placed on the glass floor plates. The flies began to feed almost immediately. It was noticed that the flies approached the drops and inserted their proboscides, but in many cases did not touch the drops with their legs. Occasionally the anterior legs were placed on the drops, but even then in many instances they did not appear to be soiled. If the drops were too close together, or were irregular, then the feet often became soiled. Occasionally a fly would fall into a drop and subsequently drag itself about the plate spreading the syrup and causing other flies which walked over the wet areas to soil their feet.

If undisturbed a fly usually becomes gorged within a minute or less. Previous to feeding, the ventral surface of the abdomen of a hungry fly when viewed from the side is slightly concave. Immediately after feeding, the anterior half of the abdomen is greatly distended while the posterior half may still remain concave. This is due to the fact that the greater part of the food is first taken into the crop, which occupies the anterior portion of the abdomen, and greatly distends that organ. Plate V., Fig. 10, represents the side view of an average unfed fly (× 7) and Plate V., Fig. 11, the side view of a fly immediately after feeding on syrup. In the latter case the distention of the crop, which can be plainly seen, was so great that the lower portions of the tergal abdominal plates, which usually overlap each other, were forced some distance apart. Experiments with syrup coloured deep red with carmine or deep blue with nigrosin show very clearly the passage of the food material into the crop. As the fly feeds on carmine syrup a red area appears on the anterior portion of the ventral surface of the abdomen, and gradually

enlarges till it occupies the whole of the anterior two-thirds of the surface of the abdomen, and usually shows a more or less well-defined convex posterior margin. Plate V., Fig. 12, shows a photograph of the ventral surface of a fly immediately after feeding on carmine syrup.

On rare occasions the red area is situated near the posterior end of the abdomen, but is continued forwards to the thorax as a distinct median red line between colourless areas. In such cases

FIG. A. 

FIG. B. 

dissection shows that the crop has been displaced by the distension of the large abdominal air sacs. [Fig. A shows the usual position of the red area, and Fig. B its position when the air sacs are distended.]

Sometimes the flies continue to feed after the crop is full, and then the food passes directly into the intestine, and after a time the whole ventral surface becomes coloured.

In order to check these observations a number of flies were killed and dissected (under a Zeiss binocular dissecting microscope) at various times after feeding on carmine syrup. The dissections showed that the crop was almost invariably distended with coloured material before any was found in the intestine. If feeding had continued beyond this point coloured material was found in the upper part of the intestine, and within a short time in the lower portion also. In one series of experiments, for example, hungry flies were fed on carmine syrup and killed and dissected at short intervals.

Table 1.—*Showing the rate at which food passes from the crop into the intestine.*

Time after feeding.	No. of flies dissected.	Result.
3 minutes	1	Crop full of red fluid, but none found in ventriculus or intestine.
6 „	1	Crop full of red fluid, but none found in ventriculus or intestine.
10 „	1	Crop full of red fluid, and some just beginning to pass into the ventriculus.
15 „	1	Crop full of red fluid, and upper third of intestine red.
20 „	1	Crop full of red fluid, and upper third of intestine red.
2 hours ...	3	Crop full of red fluid, and upper third of intestine red.
	4	Crop full of red fluid, and upper half of intestine red.
	1	Crop full of red fluid, and upper three quarters of intestine red.

The rate at which the food passes from the crop into the intestine appears to vary, depending to some extent on the temperature and the nature of the food. For example, if the flies are kept in the incubator at 37° C. and fed on carmine gelatin much of the food may reach the rectal valve within an hour.

If the flies are disturbed before the crop is completely distended the contents of the crop are gradually passed into the intestine, but the organ is not completely emptied for many hours, or in some cases for days, even though no further food is given. In most examples which were dissected the crop was found nearly empty on the third day after feeding, but the intestine still contained large quantities of red material.

Flies allowed to feed to their utmost capacity on carmine syrup showed a red colour all over the ventral surface of the abdomen within an hour or two, and dissections showed that the crop and intestine, down to the anus, were distended with coloured syrup. If such flies are subsequently fed on plain syrup it is found that most of the red material from the first meal is retained in the crop and only slowly passed into the intestine. Dissections show that a considerable quantity of the carmine, though diluted, is still present in the crop, under such conditions, after several days. For example a number of flies were fed once on carmine syrup, and were subsequently given plain syrup daily.

Table 2.—*Showing the period during which coloured food may remain in the crop.*

Time after feeding.	No. of flies dissected.	Result.
24 hours	2	Crop red and distended. Intestine red throughout.
48 „	3	Crop red and distended. Intestine red throughout.
3 days	3	Crop red and distended. Intestine red throughout.
4 „	2	Crop pink and distended. Intestine red throughout.

From these experiments, of which a large number were performed at different times, it appears that in these insects the crop performs two important functions; (A) it acts, at the time of feeding, as a large receptacle, which can be filled with great rapidity, and which consequently enables a fly which is disturbed within a few seconds of commencing a meal to carry away sufficient food to live on for some days; (B) when food is abundant the crop seems to act as a reservoir in which material can be stored against a time when food may become scarce.

The habits of flies after feeding on fluids.

A number of observations were made on the habits of flies after feeding on various fluids. After gorging themselves the insects usually climb up the sides of the cage and move from place to place, frequently stopping to rub one leg against another or to clean their heads and wings by passing their legs over them. At intervals,

however, they sit still and large drops of fluid, coloured red or blue, if the food has consisted of carmine or nigrosin syrup, or opaque and white if it has consisted of milk, exude from the tips of their proboscides. These drops gradually enlarge until they are about equal in size to the insect's head. After a longer or shorter period the drop is slowly withdrawn or deposited on the glass. Flies are frequently observed to exude and withdraw such drops several times. If disturbed they either deposit them or withdraw them with great rapidity. When deposited on the glass, as frequently happens, the drops gradually dry and each gives rise to a round stain with an opaque centre, surrounded by a clearer zone bounded by a distinct thin, more opaque marginal ring. (*See* Plate VI., Fig. 15*b*). On watching these flies the impression conveyed is that the insects have distended their crops to an uncomfortable degree and that some of the food is regurgitated in order to relieve the distension.

Flies have often been seen to suck up the drops deposited by their companions.

Whatever may be the cause of the procedure the habit is very common after feeding on all kinds of fluids, such as milk, syrup and sputum, and the stains or "spots" left by these drops can be recognised on all surfaces on which flies naturally settle.

Flies fed on coloured syrup often regurgitate coloured fluid 24 or more hours later, though fed in the interval on plain syrup. When infected food has been given the infecting organisms are usually found in great numbers in these "spots," and moreover, as will be shown later, fluid regurgitated from the crop is used to dissolve or moisten sugar and other similar dry food materials. The importance of the habit cannot therefore be overestimated.

The term "vomit" will be used to differentiate the stains left by these drops from fæcal deposits and proboscis-marks made in half dried material.

If a fly, which has been fed on coloured syrup, is killed with chloroform and pressure made with the forceps on the thorax some of the syrup may exude from the proboscis. Further pressure on the thorax or abdomen causes the proboscis to be protruded, and occasionally a large quantity of fluid may be exuded from it. Usually, however, though the proboscis appears to be distended with fluid, very little is exuded.

Possibly some mechanism exists, near the tip of the proboscis, for preventing the expression of the fluid. If, however, the tip of the proboscis is cut off, or the head removed, the contents of the crop can easily be expressed from the cut end of the proboscis or the œsophagus, even up to five or six hours after feeding.*

2. *Semi-fluid material.*

At various time flies were allowed to feed on milk which had been spread on glass in a thin layer and allowed to partially dry, and on other materials of similar consistency. The flies walked over the areas covered by the dry milk and frequently applied their proboscides to them. In all cases the application was of some duration and fluid was often deposited by the fly on the area it was

* Severe pressure on the sides of the head may cause turbid red-coloured fluid to be exuded. This seems to be derived from the eyes.

sucking. After each application an oval depression was made in the surface, in many cases showing most beautifully an imprint of the end of the proboscis, or an oval area was completely denuded. Plate VI. Fig. 13 shows numerous imprints of flies' proboscides on three circular patches of dried milk, and Fig. 14 one of the imprints more highly magnified, on which the tracings of the pseudotracheæ at the end of the proboscis can be clearly seen. If the flies had been fed previously on carmine syrup, red patches were frequently observed at the margins of these proboscis marks, either due to the deposition of carmine which had remained on the proboscis or to the regurgitation of carmine stained material to moisten the dried milk. The latter explanation is probably the correct one in most cases, for a single fly will leave many (100 or more) carmine-stained proboscis marks, and moreover carmine stains are more common when the layer of milk is rather dry, and requires more fluid to moisten it, than when it is less dry. In one experiment, made two hours after feeding on carmine syrup, half the proboscis marks showed carmine stains, and in another made 22 hours after feeding several of them showed carmine stains.

It was also frequently noticed that flies which had the opportunity of feeding on either fluid or partially dried milk often chose the drier portions. Under natural conditions they can often be seen sucking the dried remains near the top of a milk jug.

If flies are carefully observed under natural conditions, or in captivity in a cage, it is seen that they are constantly applying their proboscides to the surfaces over which they are walking, apparently attempting to suck up nutritive material. Under suitable conditions the imprints of their proboscides can often be made out.

3. *Soluble Solids.*

Flies will feed readily on crystals of brown sugar. The mode of feeding can be very accurately watched by placing one or two flies in a small cage with a crystal of brown sugar on the bottom. The cage may be easily so arranged that the lens of a Zeiss binocular microscope can be focussed on the sugar. The oral lobes of the proboscis are very widely opened and closely applied to the sugar. Fluid seems to be first deposited on the sugar and then strong sucking movements take place. When the proboscis is moved from one spot a depression in the sugar is observed, and, if the fly has been previously fed on carmine, red stains round its margin are often seen. In a number of experiments carmine stains were noticed on sugar 60, 80 and 90 minutes and even five hours after feeding on carmine.

Infection experiments described later seems to prove that in the case of flies recently fed on syrup the fluid is mainly liquid regurgitated from the crop. When the crop is empty saliva alone is probably made use of.

A fly was very carefully watched sucking an apparently quite dry layer of sputum. It put out large quantities of fluid from its proboscis and seemed to suck the fluid in and out alternately until a fairly large area was quite moist. Then as much as possible was sucked up and the fly moved on to another place.

4. *Defæcation.*

Flies which have access to abundant food defæcate frequently. The fæces, consisting of thick brownish or yellowish semi-fluid material, are deposited in single masses and quickly dry, forming opaque raised rounded stains. Occasionally the stains are pear-shaped. At ordinary temperatures flies fed on coloured syrup do not deposit coloured faeces within two hours. The faecal stains can be usually distinguished without difficulty from " vomit " stains.

Three different types of marks or " spots " have therefore to be distinguished ; (1) fæcal deposits, round, opaque, often raised and yellowish, brownish or whitish in colour ; (2) " vomit " stains, round, with a small opaque centre and clear peripheral portion, bounded by a darker zone, and (3) proboscis-marks left on half dried material.

The extraordinary number of deposits, both fæcal and vomit, left by well-fed flies, can be judged from a small number of experiments which were made with the object of ascertaining the number of deposits (fæcal and vomit) produced. (Plate VI., Fig. 15.)

In the first series (A) 10 flies were given a single feed of milk. When all had fed they were transferred to a fresh cage. At intervals the flies were again transferred to other fresh cages and the deposits in the old cages counted. In the second series (B) the deposits of 11 flies were counted in the same way, but milk was always present in the cage so that the flies could feed as often as they wished.

Table 3.—*To illustrate the number of deposits left by flies.*

Time after feeding.	Series A.			Series B.		
	Vomit.	Fæces.	Total.	Vomit.	Fæces.	Total.
1st hour	30	11	41	22	10	32
2nd and 3rd hours ...	13	3	16	31	9	40
4th hour	18	6	24	6	4	10
5th hour	15	9	24	12	6	18
6th–22nd hour ...	49	10	59	108	16	124
	125	39	164	179	45	224

Each fly in series (A) produced an average of 16·4 " spots," and in series (B) of 20·4. In another experiment 10 flies which had been given one feed of milk, produced in nine hours 209 (191 vomit and 18 fæces) deposits, and in the complete 24 hours 307 (282 vomit and 25 fæces deposits) or an average of 30·7 " spots " per fly.

No doubt the rate at which flies produce deposits depends on several factors, such as the temperature and the form of food, etc., but only a few experiments on this subject were made. Flies are more lively in hot weather or when placed in a warm incubator. That the kind of food exerts a considerable influence is shown by the following experiment. Three lots of flies were fed on syrup, milk and soutum respectively for several days. Those fed on syrup

produced an average of 4·7 deposits per fly per day, those fed on milk 8·3 and those fed on sputum 27·0. In the latter case the fæces were much more voluminous and liquid than usual and in fact the flies seemed to suffer from diarrhœa.

Summary of feeding experiments.

Musca domestica feeds readily on various liquids such as syrup, milk and sputum. Provided the food is supplied in the form of well separated, discrete drops the flies do not usually appear to soil their legs. When undisturbed the flies gorge themselves in half a minute or less. The fluid first passes into the crop, which becomes distended, and if the food is coloured its contour can be seen through the ventral surface of the abdomen. Under ordinary conditions the fluid begins to pass into the ventriculus within 10 minutes and in two or three hours coloured material can be found throughout the intestine. At high temperatures it passes more rapidly. The crop, however, is not completely emptied for many hours. Sometimes flies go on feeding after the crop is full and then the food passes directly into the ventriculus and intestine. If flies are allowed constant access to food coloured material from the first meal remains in the crop for many days.

The crop therefore seems to act as a large receptacle which can be filled with great rapidity so that flies can obtain within a few seconds sufficient nourishment to keep them alive for several days. When food is abundant the crop acts as a reservoir in which surplus food is stored for use if necessity arises.

After feeding on liquid food flies habitually exude drops of fluid from their proboscides. Sometimes these drops are sucked up again and sometimes deposited on the surface on which the flies are walking. These deposits, which have been spoken of as "vomit," dry and produce round marks with an opaque centre and rim and an intervening less opaque area.

If allowed to feed on half dried materials the flies first moisten with vomit or saliva a small area and then suck it dry. In so doing they usually leave oval depressions, often exhibiting most beautifully the markings on the proboscis, or clear areas. If the flies have previously fed on coloured syrup these proboscis marks often show traces of pigment.

When feeding on sugar small areas are moistened, either with saliva or, in the case of flies fed on fluids, with vomit. Traces of pigment are often found on the sucked areas when the flies have previously been fed on coloured syrup.

Flies which have access to abundant food leave numerous "spots" (vomit and faeces). The rate of deposition seems to vary with the kind of food and the temperature.

D.—Infection Experiments.

The first infection experiments with *B. typhosus* and *B. enteritidis* (*Gaertner*) were carried out by feeding large numbers of flies confined in gauze cages, similar to those previously described, on infected syrup. In planning these experiments it seemed reasonable to suppose that only a certain proportion of flies would feed on the

syrup and become infected, and that consequently a large number of flies would be required for each experiment. Subsequent experience, however, has shown that almost every fly becomes infected and that in consequence the experiments can be satisfactorily conducted on a smaller scale.

(1.) *Experiments with B. typhosus.*

A large number of flies were allowed to feed on syrup infected with *B. typhosus* which was left in the cage for eight hours. After that time the infected material was removed and the flies fed on plain syrup. Twenty-four hours after the introduction of the infected syrup, plates of Drigalski-Conradi medium were inoculated with an emulsion made from the intestinal contents of five flies. Eight flies were allowed to walk on plates, and the fæces of eight flies were emulsified and sown on similar plates. From all these plates *B. typhosus* was isolated. Forty-eight hours after infection eight flies were removed from the cage and allowed to walk over plates. They were afterwards killed and plates were sown with emulsions of their intestinal contents, and of the fæces deposited by them. From all these plates *B. typhosus* was isolated. Three days after infection the emulsified intestinal contents of six flies were sown, but *B. typhosus* was not isolated, the plates being overgrown with acid-forming colon-like organisms. Four days after infection eight flies were allowed to walk over plates, and emulsions of their intestinal contents and fæces were cultivated. *B. typhosus* was isolated from the plate sown with intestinal emulsion. On the fifth day after infection similar cultures were made from eight flies, but attempts to isolate *B. typhosus* from them failed. On the sixth day an emulsion of the intestinal contents of the last ten remaiuiug flies was made and inoculated on to plates. From these *B. typhosus* was isolated.

Table 4.—Showing the results of infection experiments with B. typhosus.

Time after infection.	Results of cultures made			Number of flies employed.
	By allowing flies to walk over plates.	From intestinal contents.	From emulsions of fæces.	
24 hours	+	+	+	8, 5, 3 respectively.
48 „	+	+	+	8
3 days	—	0	—	6
4 „	0	+	0	8
5 „	0	0	0	8
6 „	—	+	0	10

In this and all other Tables + indicates that the organism was isolated from the cultures, 0 that the organism was not isolated, and — that no experiment was made.

In several instances suspicious colonies were seen but isolation failed. A positive result was recorded only when the suspicious organisms were separated in pure culture and grown on agar, gelatin, broth, milk, and potato, and in peptone water containing glucose, saccharose, lactose, dulcite and mannite. They were only identified as *B. typhosus* when they behaved in every way like the stock typhoid culture with which the syrup was infected. For control purposes cultures were made from normal flies caught in various places, but no *B. typhosus-like* bacilli were isolated.

It appears from these experiments that *B. typhosus* may remain alive in the intestinal canal of the fly for at least 6 days, and that flies may infect plates on which they walk for at least 48 hours after infection.

Previous experiments.

Abstracts dealing with the work of Firth and Horrocks (1902), of Hamilton (1903), and of Ficker (1903), will be found on p. 27 of the 2nd Report to the Local Government Board on Flies as Carriers of Infection, N.S., No. 16, 1909.

Some very interesting observations and experiments have recently been published by Faichnie (1909). He investigated a small outbreak of typhoid at Ramptee, and, after excluding all other sources, was obliged to suspect the flies. They were not very numerous. About 40 were collected, 20 each from the verandahs of the artillery and infantry kitchens. "Twelve flies from the artillery lines were mashed up in sterile normal salt solution and a drop plated, with the result that *B. typhosus* was separated. This bacillus was agglutinated by a solution of 1–10,000 of a specific *B. typhosus* serum" and gave the usual reactions on various media. "Also 12 flies from the infantry kitchen were treated as follows :— Each was transfixed with a sterile needle, and passed two or three times through a flame, until the legs and wings were scorched ; they were then put into normal salt solution and stirred without breaking with a glass rod. One c.c. of this solution was seeded into McConkey broth which remained unchanged thereby showing the absence of *B. typhosus* on the legs and wings after burning. After this the flies were mashed up and a drop of the fluid plated. *B. typhosus*, as above, was again found, thereby demonstrating that the bacillus was present in the intestine, but not on the legs."

Summing up he says—"Experience seems to show that infection conveyed by flies' legs, natural though it may appear to all from experiments carried out to prove its possibility, is not a common nor even a considerable cause of enteric fever. On the other hand infection by the excrement of flies bred in an infected material explains many conclusions previously difficult to accept. In a word, it is the breeding ground that constitutes the danger, not the ground where the flies breed."

The cultures which have just been described were sent to Lieutenant-Colonel Semple, Director of the Central Research Institute, Kasauli, who stated that they were undoubtedly *B. typhosus*. Amongst other tests he immunised rabbits with these cultures and found that their serum agglutinated stock cultures of *B. typhosus* in high dilutions.

In a later paper (p. 675) the author says :—" Since writing my first paper on this subject, I have found *B. typhosus* in flies from Sehore, once ; from Kamptee, twice ; from Nasivatad, once in flies from the bungalow of an officer who had enteric fever, and once from flies in the Officers' Mess there ; from Nowgong, twice, once in the flies from the Royal Artillery Coffee Shop, and again in flies from the trenching ground, making a total of nine in three months. Except those from Nasivatad, the flies were always flamed before examination, and a control of the washed flies was taken before crushing, so there is no doubt the bacillus was actually in the interior of the fly, probably in the intestine.

The author further carried out some highly interesting breeding experiments (p. 672). " On August 12th, 1909, three ounces of fæces, containing *B. typhosus*, were thrown into a box of earth, and covered with wire gauze and about 30 flies were let loose inside. These flies all died in a day or two, but on August 26th, 14 days later one fly hatched ; on August 27th, 12 flies were hatched ; on this same day, after the flies were hatched, the box of earth was replaced by an earthenware plate which had been previously washed in a solution of 1 in 500 perchloride of mercury ; sugar and water as food in separate porcelain saucers were also introduced, and the wire cover was changed for a bell-shaped mosquito net. On August 26th one fly, one day old, was transfixed with a red hot needle after chloroforming it, flamed and put into a bottle of sterile salt solution. It was shaken up in 1 cc. of the solution put into McConkey broth, which remained unchanged for 48 hours. After this the fly was crushed with a sterile glass rod and a drop plated ; *B. typhosus* was found." Four other flies one day old gave the same results, and two flies 6 days old and two flies 9 days old gave the same results. " On September 10th two flies 13 days old were put into a dry sterile bottle and left for 24 hours ; they were then removed, and salt solution was poured into the bottle, and from this solution of excrement *B. typhosus* was obtained." The two flies were treated as the previous ones had been and *B. typhosus* was obtained. From one fly 16 days old and from its excrement *B. typhosus* was also obtained, but not from another. " From the foregoing experiment it will be seen that out of the 13 flies bred from a typhoid stool at least 6 contained *B. typhosus* in their intestines ; and the bacillus was recovered from the intestines and excrement of a fly 16 days old."

(2.) *Experiments with B. enteritidis* (*Gaertner*).

An emulsion of an agar culture of *B. enteritidis* (*Gaertner*) in syrup was placed in a gauze cage containing a large number of flies. Eight hours later the emulsion was removed and plain syrup substituted. Each day a certain number of flies were caught in a large test tube ; some were allowed to walk over Drigalski-Conradi plates, and others were killed and their intestines dissected out and emulsified and sown on similar plates. The fæces deposited on the test tube were also emulsified and sown on plates.

The results of these experiments are given in the following table :—

Table 5.—*Showing the results of infection experiments with B. enteritidis (Gaertner).*

Time after infection.	Plates from		
	Intestinal contents.	Fæces.	Flies allowed to walk over plates.
24 hours	+ (11)	—	0 (3)
48 „	0 (17)	0	+ (7)
3 days	0 (15)	0	+ (5)
4 „	+ (22)	0	+ (5)
5 „	+ (9)	—	+ (6)
6 „	+ (12)	—	+ (4)
7 „	+ (8)	—	+ (3)
8 „	0 (7)	—	0 (6)

+ indicates that B. enteritidis was isolated and proved by cultures on suitable media, including sugars; 0 that the suspicious colonies isolated did not turn out to be B. enteritidis and — that no cultures were made. Some of the plates were completely overgrown with colon-like organisms. The figures in brackets indicate the number of flies used in each experiment.

On the 7th day six flies (I.–VI., Table 6) were captured and killed. The legs and wings were removed with sterile forceps and plated separately. After flaming, the bodies were dissected and the crops and intestines isolated, separate cultures being made from the contents of each. The head was also removed and separately cultivated. On the 8th day five flies (VII.–XI.) were treated in the same way. The results of these experiments are given in the following table :—

Table 6.—*Showing the results of further experiments with B. enteritidis.*

No. of Fly.	Cultures from											
	Legs.						Wings.		Head.	Crop	Intestines.	——
	1.	2.	3.	4.	5.	6.	1.	2.				
I. ...	0	0	—	—	—	—	0	0	0	0	—	Flies dissected on the 7th day after infection.
II. ...	0	0	0	0	0	0	0	0	+	++	—	
III. ...	0	0	0	0	0	0	0	0	+	++	+	
IV. ...	0	0	0	0	0	0	0	0	+	+	+	
V. ...	+	0	0	0	0	0	—	0	0	—	0	
VI. ...	0	0	0	0	0	0	0	0	0	—	0	
VII. ...	0	0	0	0	0	0	0	0	—	0	0	Flies dissected on the 8th day after infection.
VIII. ...	0	0	0	0	0	0	0	0	0	+	0	
IX. ...	0	0	0	0	0	0	0	0	0	0	0	
X ...	0	0	0	0	0	0	0	0	0	0	0	
XI. ...	0	0	0	0	0	0	0	0	0	0	0	

++ indicates that numerous colonies of *B. enteritidis* were found.

These experiments show that *B. enteritidis* may be present in the contents of the crops and intestines of flies for at least 7 days after infection. Flies can infect plates over which they walk for some days in spite of the fact that the organisms can seldom be isolated from their legs (once in 32 cultures). When walking over plates flies constantly place their proboscides on the medium and in most cases leave imprints on its surface. The colonies develop round these marks. The infection of the plate is therefore probably due, in large measure, to inoculation by the flies' proboscis.

It is not improbable that by means of more careful and extensive experiments *B. enteritidis* might be isolated for even longer periods, since several of the plates in this series gave negative results owing to being overgrown by *B. coli*-like organism.

Previous experiments.

The only observer who seems to have worked with an organism belonging to this group is FAICHNIE (1909, p. 674). He carried out similar breeding experiments to those described with *B. typhosus*. "A second series of experiments was carried out with the fæces of a man suffering from paratyphoid fever (*B. paratyphosus A*) the diagnosis having been made by a blood culture. On August 22nd two ounces of liquid fæces, containing *B. paratyphosus A*, were put into a box of earth and about 30 flies allowed to feed on it; as the flies had no water given to them they died in a day or two. On September 1st one fly hatched out; on September 3rd 12 flies were seen. On the same date the earth was replaced by a plate as before. On September 1st one fly, one day old, was examined; the McConkey control was negative; and after being flamed and crushed *B. paratyphosus A* was obtained. On September 3rd four flies each one day old, were examined; the McConkey control was negative; and from the crushed flies *B. paratyphosus A* was separated." On September 10th 3 flies, each seven days old, were put through the sterile bottle test, and from it *B. paratyphosus A* was obtained from the crushed flies. On September 13th one fly, 10 days old, was examined, but the bacillus was not recovered. Two other flies, also 10 days old, were examined and *B. paratyphosus A* was recovered."

Later Infection Experiments.

As a result of the experience gained from the experiments which have just been described, the method of procedure was modified. Smaller numbers of flies were used and kept in the glass cages which have been alluded to. Infected material was only supplied to them for 15 minutes or less, and the cages were changed daily. In prolonged experiments they were fed, every day, with plain syrup or other food. For cultural purposes two or three flies were caught in a large test-tube and killed by chloroform vapour. The legs, wings, and heads were cut off and separately inoculated on differents parts of an agar plate. In many cases fluid was also expressed from the proboscis by means of pressure on the head and separately cultivated. The body, after being placed in alcohol, or

singed in the flame, was dissected, under a Zeiss binocular microscope, and the contents of the crop and intestines separately inoculated. The mounted Hagedorn needles and other instruments used for dissection were sterilised in the flame. After a little experience it is not difficult to dissect out the entire crop and sow its contents, and to subsequently remove the intestine without contamination with the crop contents. The agar plates on which the cultures were generally made were prepared and dried for a few minutes in the incubator at 37° C., and the legs, wings, head, and crop, and intestinal contents, &c. from one fly inoculated at different places. The spots where the crop and intestinal contents had been placed were marked by blue rings made with a glass pencil on the back of the plate, and when, as was often found possible, the organs of several flies were inoculated on one plate, those belonging to each fly were surrounded by a blue line and numbered.

Plate VII., Fig. 17 illustrates a plate, before cultivation. inoculated with the organs of four flies infected with anthrax. Figure 18 shows the same plate after 24 hours' incubation.

(3.) *Experiments with B. prodigiosus.*

A large number of preliminary experiments were carried out with *B. prodigiosus*, which was selected for the purpose because it is an organism which is easily cultivated and identified on plate-cultures. Moreover it seemed likely that the results would give some information as to the length of life of other non-spore-bearing and less easily recognizable organisms under similar conditions, and afford some indications of the best methods of procedure in making investigations on them.

(*a.*) *Experiments on the duration of life of B. prodigiosus on the exterior, and in the alimentary canal of flies.*

The following table summarises the results of four series of experiments, done at different times, on the length of life of *B. prodigiosus* on the feet and wings, and in the alimentary canal. In each case syrup infected with *B. prodigiosus* was placed for a few minutes in a cage containing hungry flies. After feeding, the flies were transferred to fresh cages, where, if the experiment was prolonged, they were fed daily with plain syrup. At intervals, specimens were caught and dissected, and their legs, wings, heads, and the contents of their crops and intestines inoculated on to agar plates.

Table 7.—Showing the length of life of B. prodigiosus on the exterior and in the alimentary canal of flies.

Time after infection.	Cultures from										
	Legs.						Wings.		Head.	Crop.	Intestine.
	1.	2.	3.	4.	5.	6.	1.	2.			
1 minute ...	—	—	—	—	—	—	—	—	++	+++	+
30 „ (1)	+	+	+	+	—	—	—	—	++	0	0
30 „ (2)	0	0	0	0	0	0	0	0	++	+++	+++
30 „ (3)	0	+	+	0	0	0	0	0	+	+++	+++
30 „ (4)	0	0	0	0	0	0	0	0	++	+++	+++
30 „ (5)	+	+	+	0	0	0	+	0	++	+++	+++
60 „ ...	+	+	—	—	—	—	+	+	++	+++	+++
90 „ (1)	+	+	+	+	0	0	+	+	++	++	++
90 „ (2)	0	0	0	0	0	0	0	0	+	+++	+++
2 hours ...	+	+	+	0	0	0	0	0	0	0	0
2·5 „ ...	0	0	0	0	0	0	0	0	0	++	++
3 „ ...	0	0	0	0	0	0	0	0	+	+++	+++
3·5 „ ...	+	0	0	0	0	0	0	0	+	+++	+++
4 „ ...	0	0	0	0	0	0	0	0	—	—	—
5 „ ...	0	0	0	0	0	0	0	0	—	—	—
5·5 „ ...	+	+	+	0	0	0	+	+	++	+++	+++
12 „ (1)	+	+	+	+	+	0	+	+	+	+++	+
12 „ (2)	+	+	+	+	+	0	+	0	+	++	—
12 „ (3)	+	+	0	0	0	0	0	0	+	+++	—
12 „ (4)	0	0	0	0	0	0	0	0	+	+++	+
12 „ (5)	0	0	0	0	0	0	0	0	+	+++	++
12 „ (6)	0	0	0	0	0	0	0	0	+	+++	++
18 „ (1)	+	+	+	+	0	0	0	0	++	+++	+++
18 „ (2)	+	0	0	0	0	0	0	0	++	+++	+++
18 „ (3)	0	0	0	0	0	0	0	0	++	+++	+++
18 „ (4)	0	0	0	0	0	0	0	0	+	+++	++
24 „ (1)	0	0	0	0	0	0	0	0	++	+++	++
24 „ (2)	0	0	0	0	0	0	0	0	+	—	—
24 „ (3)	+	0	0	0	0	0	0	0	+	+++	++
24 „ (4)	0	0	0	0	0	0	0	0	+	++	++
24 „ (5)	+	+	0	0	0	0	0	0	+	+++	++
24 „ (6)	0	0	0	0	0	0	0	0	—	+++	+
24 „ (7)	0	0	0	0	0	0	0	0	+	++	+
29 „ (1)	+	0	0	0	0	0	0	0	+	+++	++
29 „ (2)	0	0	0	0	0	0	0	0	+	+++	++
30 „ (1)	0	0	0	0	0	0	0	0	+	+++	++
30 „ (2)	0	0	0	0	0	0	0	0	+	++	++
52 „ (1)	0	0	0	0	0	0	0	0	+	+	+++
52 „ (2)	0	0	0	0	0	0	0	0	0	0	0
60 „ (1)	0	0	0	0	0	0	0	0	+	++	+++
60 „ (2)	0	0	0	0	0	0	0	0	+	++	+++
3 days (1)	0	0	0	0	0	0	0	0	0	0	0
3 days (2)	0	0	0	0	0	0	0	0	+	++	+
3 days (3)	0	0	0	0	0	0	0	0	0	+++	+
3 days (4)	0	0	0	0	0	0	0	0	+	+++	0
3 days (5)	0	0	0	0	0	0	0	0	+	++	+
3 days (6)	0	0	0	0	0	0	0	0	0	++	+
3 days (7)	0	0	0	0	0	0	0	0	+	++	+

Time after infection.		Cultures from										
		Legs.						Wings.		Head.	Crop.	Intestine.
		1.	2.	3.	4.	5.	6.	1.	2.			
4 days	(1)	0	0	0	0	0	0	0	0	+	+++	++
	(2)	0	0	0	0	0	0	0	0	0	++	+++
5 „	(1)	0	0	0	0	0	0	0	0	0	+	+ (3)
	(2)	0	0	0	0	0	0	0	0	+	+++	++
	(3)	0	0	0	0	0	0	0	0	0	0	+ (1)
8 „	(1)	0	0	0	0	0	0	0	0	+ (1)	++ (40)	++ (31)
	(2)	0	0	0	0	0	0	0	0	+	0	+ (3)
	(3)	0	0	0	0	0	0	0	0	0	0	+
9 „	...	0	0	0	0	0	+ (1)	0	0	0	0	0
11 „	...	0	0	0	0	0	0	0	0	+ (1)	—	+ (4)
12 „	(1)	0	0	0	0	0	0	0	0	0	—	0
	(2)	0	0	0	0	0	0	0	0	0	0	+ (2)
14 „	...	0	0	0	0	0	0	0	0	0	—	+ (5)
15 „	...	0	0	0	0	0	0	0	0	0	—	+ (5)
16 „	...	0	0	0	0	0	0	0	0	0	—	0
17 „	...	0	0	0	0	0	0	0	0	0	—	+ (13)
18 „	...	0	0	0	0	0	0	0	0	0	0	0
19 „	...	0	0	0	0	0	0	0	0	0	0	0
20 „	...	0	0	0	0	0	0	0	0	0	0	0
21 „	...	0	0	0	0	0	0	0	0	0	0	0

+ indicates a few colonies of *B. prodigiosus*, ++ several colonies, and +++ numerous colonies. The numbers in brackets after the results indicate the number of colonies found.

It is evident from the above table that *B. prodigiosus* may remain alive on the legs and wings for at least 18 hours after feeding. Exceptionally it may remain alive longer. It is present, in large numbers, in the contents of the crop and intestine and on the proboscis for four or five days. After this time its numbers gradually diminish, cultures after 17 days yielding negative results.

(*b.*) *Experiments to determine whether B. prodigiosus multiplies in the crop.*

A fine capillary was drawn out of thermometer tubing and marks scratched on it with a file, one about half an inch and the other about two inches from the end. Flies were fed on a dilute emulsion of *B. prodigiosus*. Some of this emulsion was drawn up to the first mark, and water to the second mark to dilute it. The fluids were mixed and the mixture sown on an agar plate. At intervals flies were killed and their crops dissected out. Some of the fluid from the crop was drawn up to the first mark, diluted and sown. Between each culture the pipette was sterilised with alcohol and washed and dried. Approximately the same number of colonies

grew in cultures made from the emulsion and from the crop contents of flies dissected one, four, five and a half and eight hours after feeding. About half the number of colonies grew in cultures made from the crop contents of flies dissected 24 and 31 hours later, which had been allowed to feed once in the interval on plain syrup.

In another similar experiment the colonies were counted.

A fly was dissected 45 minutes after feeding and 4,500 colonies were counted.

A fly was dissected 75 minutes after feeding and 5,490 colonies were counted.

A fly was dissected 2·75 hours after feeding and 4,900 colonies were counted.

A fly was dissected 5·5 hours after feeding and 4,098 colonies were counted.

A fly was dissected 24 hours after feeding and 247 colonies were counted.

A fly was dissected 3 days after feeding and 10 colonies were counted.

These experiments seen to indicate that in the case of *B. prodigiosus* multiplication does not take place in the crop.

(*c.*) *The infection of agar plates by living flies.*

In each of the following experiments two or more flies which had previously fed on syrup infected with *B. prodigiosus* were allowed to walk for 30 minutes over the surface of agar plates. For the first few minutes the flies generally walked rapidly over the surface, but subsequently they frequently stopped, and applying their proboscides apparently sucked the agar. In some cases, especially if the agar was not too dry, oval marks were left where the probosides were applied, and it was round these marks that the prodigiosus colonies grew. The following table shows the result of these experiments.

Table 8.—*Showing the results of experiments with living flies allowed to walk over the surface of agar plates.*

Time after feeding on infected syrup.	No. of flies allowed to walk on plate.	Result.
1 day	2	About 600 colonies of *B. prodigiosus*.
3 days	2	Numerous colonies of *B. prodigiosus*.
4 ,, ...	3	,, ,, ,,
5 ,, ...	3	,, ,, ,,
6 ,, ...	6	Several ,, ,,
7 ,, ...	3	,, ,, ,,

(*d*) *Experiments on the infection of sugar.*

Flies were allowed to feed on syrup infected with *B. prodigiosus* for 15 minutes, and were then removed to fresh cages. At various times one or two crystals of brown sugar were placed in the cage and the flies allowed to suck them for some time. The crystals were then taken out and dissolved in a drop of water, and the solution sown on agar. The results of three series of experiments of this kind are incorporated in the following table:—

Table 9.—*Showing results of allowing infected flies to feed on sugar.*

Time after infection when sugar given.		Results.	
3 hours	*B. prodigiosus* cultivated.	Numerous colonies.	
3·5 „	„	„	100 colonies.
4 „	„	„	few „
4·5 „	„	„	22 „
5 „	„	„	30 „
5·25 „	„	„	1 „
5·5 „	„	„	many „
7·75 „	„	„	50 „
20 „	„	„	2 „
32 „	„	„	6 „
42 „	„	„	8 „
46 „	„	„	1 „
56 „	„	not cultivated.	
66 „	„	cultivated.	3 colonies.
3 days	„	not cultivated.	
4 „	„	„	
5 „	„	„	
6 „	„	„	
7 „	„	„	
8 „	„	„	

From these experiments it appears that flies are able to infect sugar for at least two days after feeding on an emulsion of *B. prodigiosus* in syrup.

(*e.*) *The period during which infected fæcal material is deposited.*

Several experiments to ascertain the period during which infected fæcal material may be deposited were conducted in the following way. Flies were allowed to feed on syrup infected with *B. prodigiosus* for 30 minutes, and then transferred to fresh cages. At various intervals they were again transferred to fresh cages, and the fæces left in the old cages emulsified in water, and the emulsions sown on agar.

Table 10.—*Showing the period during which infected fæces are deposited.*

Time after infection when fæces collected.	Result.
2 hours ...	*B. prodigiosus* cultivated from 3 out of 4 deposits.
3 „ ...	„ „
4 „ ...	„ „ from 3 out of 5 deposits.
6 „ ...	„ „
8 „ ...	„ „
10 „ ...	„ „ from 3 out of 5 deposits.
18 „ ...	„ „ from 6 out of 8 deposits.
26 „ ...	„ „
36 „ ...	„ not cultivated, from 5 deposits.
48 „ ...	„ cultivated from 3 out of 6 deposits.
3 days ...	„ not cultivated, from 5 deposits.
4 „ ...	„ cultivated from 3 out of 7 deposits.
5 „ ...	„ not cultivated.
6 „ ...	„ cultivated. One colony in cultures from 8 deposits.
7 „ ...	„ not cultivated.
8 „ ...	„ „
9 „ ..	„ „

These experiments show that heavily infected fæcal material may be deposited for at least two days after infection.

(*f*) *The influence of various kinds of food on the period during which infected fæcal material is passed.*

As it had previously been found that the rate of deposition of fæces depended to some extent on the kind of food, an experiment was made in order to ascertain whether the period during which the fæces continued infective was influenced by the food. Different batches of flies were allowed to feed for 15 minutes on syrup, milk and sputum infected with *B. prodigiosus* and then transferred to fresh cages. Every day the cages were changed and the flies fed on non-infected syrup, milk and sputum respectively. The fæcal material present in the old cages was emulsified in water and the emulsion was sown on agar. The following table gives the results of these experiments.

Table 11.—*Showing the influence of the food on the infectivity of the fæces.*

Time after infection when fæces collected.	Cultures from the fæces of flies fed on		
	Milk.	Syrup.	Sputum.
1 day	+	+	+
2 days...	+	+	+
3 „	+	+	0
4 „	+	+	0
5 „	+	0	0
7 „	+	0	0
8 „	0	0	0
9 „	0	0	0
13 „	0	—	—

This table shows that *B. prodigiosus* cannot be cultivated from the fæces of flies fed on sputum after 48 hours. It was noticed that the fæces of these flies were much more voluminous than those of the flies fed on milk or syrup. The fæces of the flies fed on milk were infective for 7 days and those of the flies fed on syrup for only 4 days.

To ascertain how long *B. prodigiosus* is capable of surviving in various fluids dried on glass, small drops (about the size of the fæcal deposits) of the syrup, milk and sputum used to infect the flies and of an emulsion in water were placed on glass and cultures made from them at intervals. It was found that *B. prodigiosus* could no longer be recovered from the dried watery emulsions after 18 hours. They were still present in small numbers in the milk and syrup drops after 28 hours. In the sputum emulsions similarly treated they were present after 3 days in considerable numbers.

(*g*) *The infection of fresh flies from the deposits of infected flies.*

A number of experiments were made to ascertain whether clean flies became infected if placed in cages lately occupied by infected flies. (*a*) A number of flies were fed on syrup infected with *B. prodigiosus*. One hour after feeding they were transferred to a fresh cage, and allowed to remain there for two hours, and then transferred to a third fresh cage. In the second cage numerous deposits of fæces and vomit were left. Eight clean hungry flies were then put into the second cage, and immediately sucked at the deposits left by the infected flies. After being allowed to remain in the cage for various times these 8 flies were dissected and cultures made from their organs, with the following results.

Table 12.—*Showing the infection of clean flies from the deposits of infected flies.*

In infected cage for	No. of fly.	Cultures from									
		Legs.						Wings.		Head.	Intestine.
		1.	2.	3.	4.	5.	6.	1.	2.		
1·5 hours	1	0	0	0	0	0	0	0	0	+	+++
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	+ (1 colony).
	4	0	0	0	0	0	0	0	0	0	+
3·5 „	5	0	0	0	0	0	0	0	0	+	+ (3 colonies).
	6	0	0	0	0	0	0	0	0	0	0
5 „	7	0	0	0	0	0	0	0	0	0	+ 3 (colonies).
	8	0	0	0	0	0	0	0	0	+	+ 1 (colony).

These experiments show that clean flies may sometimes infect themselves from the deposits of infected flies.

(*b*) A number of flies were fed on syrup infected with *B. prodigiosus* for 30 minutes and were then transferred to a clean cage for two hours. At intervals of two hours they were transferred to fresh cages, and four to six clean flies put into the cages from which they had been removed. After remaining two hours in the contaminated cages the clean flies were killed and dissected, and cultures made from their organs.

Table 13.—*Showing the results of further experiments on the infection of clean flies from the deposits of infected flies.*

Time.	No. of fly.	Cultures from										
		Legs.						Wings.		Head.	Crop.	Intestine.
		1.	2.	3.	4.	5.	6.	1.	2.			
2 hours	1	0	0	0	0	0	0	0	0	0	+(2)	0
	2	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0
4 „	7	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	—	—	—
	9	0	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0	0
6 „	13	0	0	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	+ (2 colonies).
	16	0	0	0	0	0	0	0	0	0	0	+ (1 colony).
	17	0	0	0	0	0	0	0	0	0	0	+ (1 colony).
8 „	18	0	0	0	0	0	0	0	0	0	0	0
	19	0	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0	0
	21	0	0	0	0	0	0	0	0	0	0	0
10 „	22	0	0	0	0	0	0	0	0	0	0	0
	23	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0	0
	25	0	0	0	0	0	0	0	0	0	0	0

In this experiment the clean flies used were taken from a cage in which clean syrup had been placed at intervals. Consequently they were not very hungry, and did not suck the infected deposits as readily as did the flies in the previous experiment. Nevertheless 16 per cent. became slightly infected. In no case were the feet infected.

(*c*) In another series of experiments an attempt was made to obtain some information as to the period of time during which flies deposit material which is capable of causing infection in fresh flies

Twelve flies were allowed to feed on syrup infected with *B. prodigiosus* for 15 minutes, and then removed to a fresh cage (A). Next day they were removed to another cage (B), and three clean flies were put into cage A for four hours, and then dissected. For seven days the infected flies were transferred daily to fresh cages and clean flies put into the cages vacated by them for four to six hours and then dissected. The results of these experiments are given in the following table.

Table 14.—*Showing the results of further experiments on the infection of clean flies from the deposits of infected flies.*

Time after infection.	No. of clean flies used.	Cultures from the Legs. 1.	2.	3.	4.	5.	6.	Wings. 1.	2.	Head.	Crop.	Intestine.
18 hours	1	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0
42 „	4	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0
3 days	7	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0	0	+ (1 colony).
4 „	10	0	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0	0
5 „	13	0	0	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0
6 „	16	0	0	0	0	0	0	0	0	0	+ (1 colony).	0
	17	0	0	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0
7 „	19	0	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0	0

These experiments seem to indicate that clean flies may sometimes infect themselves from the vomit or fæces deposited by infected flies several days after infection.

(*h*) *Experiments on the infection of milk.*

Twelve flies were allowed to feed on syrup infected with *B. prodigiosus* for fifteen minutes, and were then transferred to a fresh cage. Twenty hours later some drops of milk were placed in the cage, and the flies immediately fed on it. After they had sucked up about half the milk the rest was taken up into a pipette and well

mixed. A part of the contents of the pipette was plated immediately, and the other portions plated after being kept at room temperature for three and 20 hours respectively, for the purpose of allowing *B prodigiosus*, if present, to multiply. *B. prodigiosus* was not found in any of these cultures. Cultures were made in the same manner from milk on which these flies were allowed to feed 42 hours after infection, with negative results. Similar experiments on the third day yielded a few colonies of *B. prodigiosus*, but those made on the 5th, 6th, 7th and 8th days were negative.

In another experiment 10 flies were allowed to feed on infected syrup and then transferred to a fresh cage. Two hours later a large drop of milk was placed on the floor of the cage, and when about half had been consumed the rest was drawn up into a pipette and kept at room temperature for 18 hours and then plated. Similar experiments were made 4, 4½, 6, 7, 8, 9, and 10 hours after infection, all with negative results.

In another experiment five flies were allowed to feed on infected syrup and transferred to a fresh cage. The experiment was conducted in the same way as the last, but sterile and not ordinary milk was given, 2, 4½, 5½, 7, 26, 28, 30, and 31 hours after infection. The results were negative.

It was thought that the negative results of the first experiments might have been due to overgrowth of *B. prodigiosus* by the other organisms present in the milk. The last experiment seems to indicate however that flies sometimes do not infect fluids which they suck up. Probably this is owing to the fact that the contents of the crop are not regurgitated in order to moisten the food as in the case of sugar. Fæces passed at intervals during these experiments were examined and found to be infected.

(*i*). *Experiments on the Infection of Fluids by Flies which get into them.*

Flies are frequently found drowned in milk, and it seems not unlikely that previous to death the flies may defæcate or otherwise pollute the fluid. The following experiments were made in order to test this point. A number of flies were fed on syrup infected with *B. prodigiosus*, and then removed to a clean cage. At intervals (½, 1½, 2, 3, 4, 5, 24, 28, and 30 hours) two flies were caught and placed separately in small tubes of milk and left in for one hour. Cultures were made from each of these milk tubes, one immediately after the fly was taken out, and another after the tube had been kept at room temperature for 24 hours. All the cultures were negative. The fæces passed by the flies at various times were tested and found to be infected.

Similar experiments in which peptone water was substituted for milk were also negative.

(*j*.) *Experiments on the Infection of Meat.*

Six flies were allowed to feed on syrup infected with *B. prodigiosus* and transferred to a fresh cage. At intervals (2, 3½, 6, 7, 26, 28, 30,

and 32 hours) small pieces of fresh meat were placed in the cage. The flies walked on these and eagerly sucked them. After a few minutes the pieces were removed and rubbed over the surface of an agar plate. Though numerous colonies of other bacteria developed, *B. prodigiosus* was not found.

Previous experiments by Burgess, quoted by Hart and Smith (1893) and Abel (1899) are abstracted on p. 29 of the 2nd Report to the Local Government Board on Flies as Carriers of Infection, N.S., No. 16, 1909.

Summary of experiments with B. prodigiosus.

The experiments which have been carried out in relation to the period during which *B. prodigiosus* remains alive on the legs and wings and in the crop and intestinal contents of infected flies, on the infection of sugar and agar plates by living flies, and on the time during which infected material may be deposited, are sufficiently numerous and conclusive to allow of definite statements being made on these points.

B. prodigiosus may be cultivated from the legs and wings of infected flies for 18 hours (and occasionally longer) after infection. It can be cultivated from the contents of the crop and intestine in large numbers up to 4 or 5 days, and has been found surviving in the intestine up to 18 days. There is no evidence to show that *B. prodigiosus* multiplies in the crop. Flies allowed to walk over agar plates are capable of infecting them (probably by means of material regurgitated through their proboscides) for at least 7 days. They are capable of infecting sugar for at least 2 days. Infected fæces may be deposited during several days after infection, the periods varying with the kind of food. Flies fed on milk deposited infected fæces during 7 days, those fed on syrup during 4 days, and those fed on sputum for 2 days. Clean flies may infect themselves by feeding on the deposits left by infected flies, especially if the latter have been freshly passed shortly after infection. Milk seems to be rarely contaminated by infected flies whether they merely drink it or fall into it. In the single experiment which was tried flies which walked and fed on meat did not infect it. Possibly *B. prodigiosus* is not a suitable organism for the last two experiments.

4. (*a*) *Experiments with B. tuberculosis* (*Culture*).

A large number of freshly-caught flies were allowed to feed on an emulsion of a culture of human tubercle bacilli in syrup. After feeding, the flies were transferred to a clean cage and fed daily on syrup. After varying intervals of time flies were caught and dissected. Smear preparations were made from the crop and intestinal contents and stained in the usual way for tubercle bacilli. Smears were also made from vomit and fæcal material and stained in the same way. Each of the preparations was very carefully examined for tubercle bacilli by two observers.

Table 15.—*Showing the results of an experiment with a culture of B. tuberculosis.*

Time after infection.	No. of fly.	Crop.	Intestine.	Fæces.
17 hours ...	1	+	+ (many)	—
22 „ ...	2	+	+ „	—
55 „	3	+	+ ...	+
	4	+	+ ...	
58 „ ...	5	+	+ ...	+ in 6 out of 6 samples.
78 „	6	+	+ ...	+ in 3 out of 3 samples.
	7	+	+ ...	also in vomit.
96 „	8	—	+ ...	+ in 5 out of 6 samples.
	9	—	+	
5 days...	10	0	+ (few) ...	+ in 3 out of 7 samples.
	11	0	+ „	
6 „ ...	12	0	+ (many)	+ only 1 tubercle bacillus found in several samples.
	13	—	+ (several)	
7 „ ...	14	—	+ „	—
	15	—	+ „	
8 „ ...	16	—	+ (8 found)	+ several.
	17	—	+ (2 „)	
9 „ ...	18	—	+ (several)	0
	19	—	0	
10 „ ...	20	—	+ (several)	0
	21	—	+ „	
11 „ ...	22	—	+ (2 found)	+ (1 tubercle bacillus found).
	23	—	+ (few)	
12 „ ...	24	—	+ (1 clump)	0
	25	—	+ (2 found)	
13 „	26	—	0 ...	+ 3 tubercle bacilli in 2 samples.
14 „	27	—	0	
15 „ ...	28	—	0	
	29	—	0	
	30	—	0	
	31	—	0	
16 „	32	—	+ 1 tubercle bacillus found.	

These experiments show that, under experimental conditions, tubercle bacilli, derived from a culture, may be present in the crop for three days. In the intestine, however, they may be found for much longer periods, being present in considerable numbers for at least 6 days. Subsequently their numbers diminish, but they may be discovered by careful search for 12 days or even longer. In the fæces they are numerous up to the 5th day, and occasional specimens may be found in fæcal material deposited between the 6th and 14th days after infection. A number of preparations made from the crop and intestinal contents of normal flies were examined but acid-fast bacilli were never found.

4. (*b*) *Experiment with B. tuberculosis in sputum.*

A large number of flies were allowed to feed for 30 minutes on sputum, rich in tubercle bacilli, which they took up greedily. Subsequently they were fed on non-tuberculous sputum. In other

respects the experiment was conducted in the same way as the previous one, except that the contents of the crop were not examined.

Table 16.—*Showing the results of an experiment with sputum containing B. tuberculosis.*

Time after infection.	No. of fly.	Intestine.	Fæces.
20 hours	1	+ ...	+ (few).
	2	+	
50 „	3	+ (few) ...	+ in 3 out of 6 samples.
	4	+ „	
69 „	5	+ ...	+ in 2 out of 6 samples.
	6	0	
90 „	7	+ ...	+ in 1 out of 3 samples.
	8	+	
4 days	9	+ (1 found)	0 in many samples.
	10	0	
5 „	11	0 ...	+ 1 tubercle bacillus in many samples
	12	+ (2 found)	
6 „	13	0 ...	0 in many samples.
	14	0	
7 „	15	+ (few) ...	0 „ „
	16	0	
8 „	17	0 ...	0 „ „
	18	0	
9 „	19	0 ...	0 „ „
	20	0	
10 „	21	0	
13 „	23, 24, 25	Intestinal contents emulsified and injected into a guinea-pig. The animal was kept under observation for 8 weeks. After that time it was killed and found to be healthy.	

This experiment shows that under more natural conditions tubercle bacilli may be found in the intestinal contents of flies for at least four days. The fæces passed during that period are also infected. The experiment is not, however, quite comparable with the previous one since continual feeding on sputum gives the flies diarrhœa.

Previous experiments.

Abstracts of the work of Spillman and Haushalter (1887), of Hofmann (1888), of Celli (1888), of Hayward and of Lord (1904), and of Buchanan (1907), will be found on p. 23 of the 2nd Report to the Local Government Board on Flies as Carriers of Infection, N.S. No. 16, 1909.

5. (*a*) *Experiment with B. anthracis in blood* (Non-spore bearing).

Twenty-four flies were placed in a cage for one hour together with the body of a mouse just dead of anthrax. The latter had been opened so that the flies could feed on the blood. The flies were then transferred to a clean cage. The next morning the cage

contained numerous red spots of vomit, and several masses of yellowish fæces. In the former *B. anthracis* was found both microscopically and by cultures. The flies were transferred daily to fresh cages and fed on syrup. At intervals specimens were caught and dissected and cultures made, on agar, from their legs, wings, heads, and crop and intestinal contents. Cultures were also made from the fæcal deposits.

Table 17.—*Showing results of experiments with B. anthracis* (Non-spore bearing).

Time after infection.	No. of fly.	Cultures from														
		Legs.						Wings.		Head.	Crop.		Intestine.		Fæces.	
		1.	2.	3.	4.	5.	6.	1.	2.		M.	C.	M.	C.		
18 hours	1	0	0	0	0	0	0	0	0	0	+	+	+	+	—	
	2	0	0	0	0	0	0	0	0	+	—	—	+	+		
24 „	3	+	+	0	0	0	0	0	0	0	—	—	+	+	+	
	4	0	0	0	0	0	0	0	0	+	+	+	—	+		
	5	0	0	0	0	0	0	0	0	—	—	—	+	+		
	6	0	0	0	0	0	0	0	0	0	—	—	+	+		
48 „	7	0	0	0	0	0	0	0	0	+	0	+	+	+	+	
	8	0	0	0	0	0	0	0	0	0	0	0	0	0		
	9	0	0	0	0	0	0	0	0	+	—	+	—	+		
	10	0	0	0	0	0	0	0	0	0	—	0	—	0		
3 days	11	0	0	0	0	0	0	0	0	0	+	+	—	+	0	
	12	0	0	0	0	0	0	0	0	0	—	+	—	+		
	13	0	0	0	0	0	0	0	0	0	0	0	—	0		
	14	0	0	0	0	0	0	0	0	0	—	0	—	+		
4 „	15	0	0	0	0	0	0	0	0	0	0	0	—	0		
	16	0	0	0	0	0	0	0	0	+	+	+	—	0	Crop full of apparently coagulated blood.	
	17	0	0	0	0	0	0	0	0	0	—	—	—	0		
5 „	18	0	0	0	0	0	0	0	0	0	—	—	—	0		
	19	0	0	0	0	0	0	0	0	0	—	—	—	0		
	20	0	0	0	0	0	0	0	0	0	+	+	—	0	„	„
	21	0	0	0	0	0	0	0	0	0	—	0	—	0		

M = Microscopic preparation. C = Culture.

This experiment shows that non-spore-bearing anthrax bacilli do not remain alive on the external parts of flies for more than 24 hours. They may, however, remain alive in the intestine for three days, and in the crop for five days, especially when partially coagulated blood remains in that organ. Film preparations made at various times from the crop and intestinal contents showed no spore-bearing forms. Plate VI., Fig. 16, is a reproduction of a photograph of a smear made from the crop of a fly (No. 11). The bacilli are present in the fæces deposited 48 hours after infection.

5. (*b*) *Experiments with anthrax spores.*

An emulsion of an old anthrax culture was made and heated to 70° C. for 15 minutes, and subsequently a number of flies were allowed to feed on it. These flies were transferred to fresh cages daily and fed on syrup. Specimens were caught and dissected at intervals. Cultures were made, on agar, from their legs, wings, heads, crop and intestinal contents and fæcal deposits. Although numerous smears were made from crop and intestinal contents at various times, no anthrax bacilli were seen microscopically, showing that the spores do not develope into bacilli in the fly.

Table 18.—*Showing the results of experiments with anthrax spores.*

Time after infection.	No. of fly.	Cultures from: Legs 1.	Legs 2.	Legs 3.	Legs 4.	Legs 5.	Legs 6.	Wings 1.	Wings 2.	Head.	Crop.	Intestine.	Fæces.	Vomit.
2·5 hours...	1	0	0	0	0	0	0	+	0	++	++	++	—	—
4 „ ...	2	+	+	+	+	+	+	+	+	++	++	++	0	+
19 „ ...	3	0	+	+	+	+	+	+	+	++	++	++	+ (3 out of 4)	+ (5 out of 5).
24 „ ...	4	+	+	+	+	+	+	+	+	+	+	++		
28 „ ...	5	+	+	+	+	+	+	+	+	+	—	++	+ (3 out of 4)	
30 „ ...	6	+	+	+	+	+	+	+	+	+	—	++	+ (8 out of 8)	+ (5 out of 5).
48 „	7	+	+	+	+	+	+	+	+	+	—	++		
	8	+	+	+	+	0	0	0	0	++	—	++		
54 „ ...	9	+	+	+	+	+	+	+	+	+	++	++		
3 days	10	+	0	0	0	0	0	+	0	+	++	++	+ (1 out of 5)	+ (1 out of 4).
	11	+	+	+	+	0	0	+	0	+	++	++		
	12	+	+	+	+	0	0	+	+	+	—	++		
4 „	13	+	+	0	0	0	—	+	+	+	+	++	+ (3 out of 5)	+ (4 out of 5).
	14	+	0	0	0	0	0	+	+	+	+	++		
5 „	15	0	0	0	0	0	0	0	0	0	++	++	+ (3 out of 7)	0 out of 3.
	16	0	0	0	0	0	0	0	0	0	++	+		
6 „ ...	17	+	+	0	0	0	0	0	0	+	++	+	0 out of 4	+ (1 out of 5).
7 „ ...	18	0	0	0	0	0	0	0	0	0	++	++	0	
8 „ ...	19	+	+	0	0	0	0	0	0	0	+	0		
9 „ ...	20	0	0	0	0	0	0	0	0	0	0	0	+ (1 out of 7)	
10 „ ...	21	+	0	0	0	0	0	+	0	0	—	+	0	
11 „ ...	22	0	0	0	0	0	0	0	0	+	0	0	0	
12 „ ...	23	0	0	0	0	0	0	0	0	0	0	0	0	
13 „ ...	24	0	0	0	0	0	0	0	0	0	0	0	—	
15 „ ...	25	0	0	0	0	0	0	0	0	0	0	0	—	
16 „ ...	26	0	0	0	0	0	0	0	0	0	+	0	—	

Cultures were also made from drops of syrup after the flies had been allowed to feed on them. Anthrax bacilli were cultivated on the 3rd, 4th, 6th, 7th, 8th, and 10th days, but not later.

This experiment shows that flies infected with anthrax spores may carry the spores on their legs and wings for at least 12 days, and that the spores are present in considerable numbers in the crop and intestinal contents for at least 7 days. The vomit and fæcal deposits contain living spores for 6 days or longer. In another

experiment, which was intended to confirm and prolong the one just described, a number of flies were allowed to feed on syrup infected with anthrax spores. After feeding they were transferred to a fresh cage and fed daily on plain syrup. After a week, specimens were caught and dissected and cultures made on agar. Unfortunately an epidemic of *Empusa muscæ* killed off these flies after 20 days.

Table 19.—*Showing the results of further experiments with anthrax spores.*

Time after infection.	No. of fly.	Cultures from: Legs. 1.	2.	3.	4.	5.	6.	Wings. 1.	2.	Head.	Crop.	Intestine.	Fæces.
7 days ...	1	+	+	+	+	+	+	+	+	—	+	+	—
8 „ ...	2	+	+	+	0	0	0	0	0	0	—	++	
12 „	3	+	+	+	+	0	0	+	0	0	+	+	
	4	+	0	0	0	0	0	+	0	0	—	+	
	5	+	+	+	0	0	0	+	0	+	—	+	
	6	+	+	+	+	0	0	+	0	+	++	+	
	7	+	0	0	0	0	0	+	0	0	0	0	
13 „ ...	8	+	0	0	0	0	0	+	+	+	+	+	+
20 „ ...	9	+	+	0	0	0	0	+	0	+	—	+	0
47 „	A	+	+	+	0	0	0	+	0	+	—	+	Dead flies picked out of the cage and kept in a bottle.
	B	+	+	+	+	0	0	+	0	+	—	0	
155 „ ...	C	+	+	+	+	+	—	—	—	—	—	+	

B. Anthracis was isolated from the cultures obtained from the legs of flies A and C and in each case found to be virulent to guinea-pigs.

This experiment confirms the last and shows that anthrax spores may remain alive on the legs and wings and in the intestinal contents for at least 20 days. Fæces passed 14 days after infection contained living spores. It further shows that the spores remain alive and virulent on the bodies of dead flies for long periods.

The experiment differs slightly from the last since the flies were kept in one cage all the time and were not transferred to fresh cages daily.

In order to ascertain how long spores will retain their vitality in dried fæces and vomit the following experiment was made. After feeding on infected material the flies used for the last experiment were placed for 24 hours in a fresh cage (A), and then removed to another. At intervals (1, 3, 4, 5, 7, 8, 12, 13, and 20 days) cultures were made both from the vomit and fæces deposited on cage A and, in every case, numerous colonies of *B. anthracis* grew. At the end of 20 days the cage was sterilised by mistake. Probably the spores would have remained alive for a much longer period.

Cultures from normal flies occasionally showed colonies somewhat resembling those of anthrax, but in most cases the chains composing the colony were much thinner than those of anthrax. In all doubtfull cases, however, subcultures were made and tested.

Previous experiments.

Abstracts of the work of earlier investigators from 1869 onwards will be found on p. 14 of the 2nd Report to the Local Government Board on Flies as Carriers of Infection. N.S. No. 16, 1909.

6. *Experiments with B. diphtheriæ (cultures).*

Two series of experiments were made with cultures of *B. diphtheriæ*. In the first, a number of flies were allowed to feed for 30 minutes on an emulsion of *B. diphtheriæ* in saliva, and were then transferred to a fresh cage. At intervals flies were killed and cultures made on transparent serum medium (Nuttall and Graham-Smith, 1908, p. 150), from their legs, wings, heads, and crop and intestinal contents.

Table 20.—*Showing results of experiments with B. diphtheriæ emulsified in saliva.*

Time after infection.	No. of fly.	Cultures from: Legs. 1.	2.	3.	4.	5.	6.	Wings. 1.	2.	Head.	Crop.	Intestine.	Fæces.
1 hour	1	0	0	0	0	0	0	0	0	+	—	+	0
	2	0	0	0	0	0	0	0	0	+	—	++	
2 hours	3	0	0	0	0	0	0	0	0	0	—	++	++
	4	0	0	0	0	0	0	0	0	+	—	++	
3 „	5	0	0	0	0	0	0	0	0	0	—	++	
4 „	6	0	0	0	0	0	0	0	0	0	0	0	
	7	0	0	0	0	0	0	0	0	0	—	0	
6 „	8	0	0	0	0	0	0	0	0	0	—	0	
	9	0	0	0	0	0	0	0	0	0	+ (1 colony).	0	+ (2 colonies).
24 „	10	0	0	0	0	0	0	0	0	0	+	0	
	11	0	0	0	0	0	0	0	0	0	++	+	
	12	0	0	0	0	0	0	0	0	0	0	0	
48 „	13	—	—	—	—	—	—	—	—	0	0	0	
	14	—	—	—	—	—	—	—	—	0	0	0	
72 „	15	—	—	—	—	—	—	—	—	0	0	0	0

In the second series of experiments the flies were allowed to feed for 1 hour on an emulsion of *B. diphtheriæ* in broth. The subsequent proceedings were the same as in the first series.

Table 21.—*Showing the results of Experiments with B. diphtheriæ emulsified in Broth.*

Time after infection.	No. of fly.	Cultures from											
		Legs.						Wings.		Head.	Crop.	Intes-tine.	Fæces.
		1.	2.	3.	4.	5.	6.	1.	2.				
5 hours	1	+	0	0	0	0	0	0	+	0	0	0	+
27 „	2	0	0	0	0	0	0	0	0	0	0	0	0
51 „	3	0	0	0	0	0	0	0	0	0	0	0	+ (2 colonies).
77 „	4	0	0	0	0	0	0	0	0	+(1)	+	+	
101 „	5	0	0	0	0	0	0	0	0	0	0	0	
	6	0	0	0	0	0	0	0	0	0	0	0	
	7	0	0	0	0	0	0	0	0	0	0	0	
116 „	8	0	0	0	0	0	0	0	0	+	+	+	
6 days	9	0	0	0	0	0	0	0	0	0	0	0	
	10	0	0	0	0	0	0	0	0	0	+	0	
	11	0	0	0	0	0	0	0	0	0	0	0	
7 „	12	0	0	0	0	0	0	0	0	0	+ (1 colony).	0	
	13	0	0	0	0	0	0	0	0	0	0	0	
8 „	14	0	0	0	0	0	0	0	0	0	0	0	
	15	0	0	0	0	0	0	0	0	0	0	0	

These experiments seem to indicate that *B. diphtheriæ* seldom remains alive for more than a few hours on the legs and wings, but may live in the crop and intestine for 24 hours or occasionally longer. The fæces passed during the first few hours are frequently infected. It is very probable that these experiments under-estimate the vitality of *B. diphtheriæ*, since in many cases film-forming bacilli overgrew the cultures.

Previous Experiments.

With regard to the dissemination of *B. diphtheriæ* by flies the only recorded work appears to be that of Smith (1898 ; cited by Dickinson, 1907), who allowed house-flies to walk over infected material and then over sterile media. Colonies of diphtheria bacilli grew on the latter.

7. *Experiments with V. choleræ.*

A number of flies were allowed to feed for one hour on a cholera culture emulsified in broth and then transferred to a fresh cage. At intervals specimens were caught and dissected, and cultures made from the head, each leg, and wing, and the crop and intestinal contents in small tubes containing peptone water, and were cultivated for 12 hours at 37° C. The contents of these tubes were then examined microscopically, and gelatin plates were inoculated from them.

Table 22.—Showing the results of gelatin plates inoculated with the Organs of Flies allowed to feed on an Emulsion of V. choleræ.

Time after infection.	No. of fly.	Cultures from: Legs. 1.	2.	3.	4.	5.	6.	Wings. 1.	2.	Head.	Crop.	Intestine.	Fæces.
5 hours	1	+	+	+	+	+	+	+	+	++	++	+++	+
30 „	2	+	0	0	0	0	0	0	0	0	0	+ (few)	+ (few).
48 „	3	0	0	0	0	0	0	0	0	0	+ (few)	+	0
77 „	4	0	0	0	0	0	0	0	0	0	0	0	0
101 „	5	0	0	0	0	0	0	0	0	0	0	0	0
6 days	6	—	—	—	—	—	—	—	—	—	0	0	—
	7	—	—	—	—	—	—	—	—	—	0	0	
	8	—	—	—	—	—	—	—	—	—	0	0	
7 „	9	—	—	—	—	—	—	—	—	—	0	0	—
	10	—	—	—	—	—	—	—	—	—	0	0	—
8 „	11	—	—	—	—	—	—	—	—	—	0	0	—
	12	—	—	—	—	—	—	—	—	—	0	0	
	13	—	—	—	—	—	—	—	—	—	0	0	

These experiments seem to indicate that the vibrios on the legs and wings soon die. Even in the crop and intestine their numbers rapidly diminish, all cultures made more than 48 hours after infection yielding negative results. Infected fæces may be passed for 30 hours.

Previous experiments.

Abstracts of the work of Tizzoni and Cattani (1886), Sawtchenko, of Simmonds and Uffelmann (1892), of Macrae (1894), of Buchanan (1897), of Tsuzuki (1904), and of Chantemesse (1905) will be found on pp. 15–17 of the 2nd Report to the Local Government Board, on Flies as Carriers of Infection. N.S. No. 16, 1909.

8. *Experiments with Danysz rat virus.*

A number of flies were allowed to feed for 1 hour on a broth culture of virus, recently recovered from the body of a rat and were then transferred to a fresh cage. A piece of bread, soaked in milk, was put into the cage daily and the flies allowed to settle and feed on it. After 1 hour the bread was removed and given to a mouse.

Table 23.— Showing the result of experiments with Danysz rat virus.

Time after infection bread given to flies.	Mouse number.	Result.
3 hours	1	Died in 4 days. Virus isolated from spleen.
24 „	2	„ 2 „
48 „	3	„ 2 „ „ „
3 days	4	„ 2 „
4 „	5	„ 2 „
6 „	6	Remained alive.
7 „	7	„ „

A mouse was also fed on bread soaked in milk containing an emulsion of fæces, passed about 48 hours after infection, scraped from the walls of the cage. The animal died in 2 days, and the organism was isolated from it.

These experiments show that flies which have fed on virus are capable of infecting food on which they settle and feed to such an extent that mice fed on it become infected.

9. *Experiments with a yeast.*

Flies were allowed to feed for 15 minutes on a culture of a yeast emulsified in syrup. They were then transferred to a clean cage and from specimens caught and dissected at intervals cultures were made on agar in the usual way. These organisms apparently do not survive for more than a few hours on the legs and wings, but can be found in cultures from the crop and intestine for at least 3 days. They are also present in the fæces passed 48 hours after infection.

Table 24.—*Showing results of experiment with a yeast.*

Time after infection.	No. of fly.	Cultures from											
		Legs.						Wings.		Head.	Crop.	Intestine.	Fæces.
		1.	2.	3.	4.	5.	6.	1.	2.				
2·5 hours	1	+	+	0	0	0	0	+	0	+	—	+	0
24 „	2	0	0	0	0	0	0	0	0	0	—	+	+
	3	0	0	0	0	0	0	0	0	0	—	+ (few)	
48 „	4	0	0	0	0	0	0	0	0	0	+ (few)	+ (few)	+ (few).
	5	0	0	0	0	0	0	0	0	0	+ (few)	+ (few)	
3 days	6	0	0	0	0	0	0	0	0	0	—	+ (few)	0
	7	0	0	0	0	0	0	0	0	0	—	+ (few)	
4 „	8	0	0	0	0	0	0	0	0	0	—	0	0
	9	0	0	0	0	0	0	0	0	0	—	0	

10. *B. enteritidis sporogenes.*

An attempt was made to carry out a series of experiments with this organism. Flies were fed on emulsions, and at intervals specimens were caught and dissected and their limbs and organs placed in small milk tubes, which were cultivated anærobically. Such tubes gave characteristic reactions for several days. It was found, however, that many normal flies contained an organism capable of giving rise to the characteristic reaction in milk. Consequently the experiments were abandoned.

Previous experiments with other pathogenic organisms.

Abstracts of the work of Yersin (1894) and of Nuttall (1897) on plague, of Castellani (1907) on Framboesia tropica (Yaws), and of Wellander (1896) on Gonorrhœa, will be found on pp. 20 and 21 of the 2nd Report to the Local Government Board on Flies as Carriers of Infection, N.S. No. 16, 1909.

Summary of infection-experiments with pathogenic organisms.

B. typhosus can be recovered from the intestinal contents of flies up to six days after infection. The flies are capable of infecting agar plates, by walking over them, for two days. *B. enteritidis* (*Gaertner*) has been recovered from the crop and intestinal contents seven days after infection. Agar plates over which the flies walked were infected up to the seventh day. In these experiments cultures were not made from the external parts. *B. tuberculosis* can be detected by microscopic examination in the crops of flies allowed to feed on cultures for three days, and in the intestinal contents for 12 to 16 days. In the intestinal contents of flies which have fed on tubercular sputum the bacilli are common up to the fourth day and can occasionally be detected up to the seventh day.

Living anthrax bacilli may be present in the intestinal contents of flies which have fed on the blood of an animal dead of anthrax for three days, and may occasionally be cultivated from the crop up to the seventh day. They were not obtained from the legs or wings after 24 hours. Spores are not developed in the body of the fly. Anthrax spores may, however, remain alive on the legs and wings and in the crop and intestines of a large proportion of infected flies for at least 20 days. Cultures can also be obtained from the bodies of dead flies for at least 115 days and proved to be virulent. The spores do not develop into vegetative forms within the body of the fly.

Diphtheria bacilli can seldom be cultivated from the legs and wings of infected flies, and rapidly diminish in numbers in the intestine. The vibrio of cholera only survives for a few hours on the legs and wings, and has not been recovered from the crop or intestine after 48 hours. Flies which have been allowed to feed on an emulsion containing Danysz rat virus are capable of infecting food on which they settle and feed to such an extent that mice subsequently fed on it become infected and die.

Tubercle bacilli can be frequently detected in the fæces and vomit deposited by flies fed on cultures during the first six days after infection, and can occasionally be detected in those deposited up to the 13th day. The fæces of flies fed on sputum contained tubercle bacilli for 90 hours. Flies fed on non-spore bearing anthrax bacilli deposited infected fæces for 24 hours, while those fed on spores deposited infected fæces for at least 14 days. The spores were found alive and virulent in fæces deposited 20 days previously. Living diphtheria bacilli may be found in fæces deposited within 51 hours of infection, and the vibrio of cholera in fæces deposited within 30 hours. *B. typhosus* has been recovered from fæces deposited 48 hours after infection.

The following table shows that non-spore-bearing organisms seldom survive more than a few hours on the legs and wings of flies. They are found for longer periods in cultures made from the proboscides and heads and are then probably derived from regurgitated food material. Most species with which experiments have been made are capable of surviving for some days in the crop and intestine, though their numbers rapidly diminish. Infected fæces are usually deposited during the first two days after infection. The only spores with which experiments were made (*B. anthracis*) survived both on the legs and wings and in the crop and intestine, and also in the fæces for many days.

Table 25.—Showing the period during which organisms were usually recovered and the last occasion after infection on which they were recovered.

Organism.	Period during which frequently recovered.						Latest occasion on which organism recovered.					
	Legs.	Wings.	Head.	Crop.	Gut.	Fæces.	Legs	Wings.	Head.	Crop.	Gut.	Fæces.
B. typhosus	—	—	—	—	2 days	2 days	—	—	—	—	6 days	2 days
B. enteritidis	—	—	—	—	1 day	0	7 days	—	7 days	8 days	7 days	0
B. tuberculosis (culture) ...	—	—	—	3 days	12 days	8 days	—	—	—	3 days	16 days	13 days
B. tuberculosis (sputum) ...	—	—	—	—	3 days	3 days	—	—	—	—	7 days	5 days
Yeast	0	0	0	0	3 days	2 days	2½ hrs.	2½ hrs.	2½ hrs.	2 days	3 days	2 days
B. diphtheriæ	0	0	2 hrs.	2 hrs.	2 hrs.	6 hrs.	5 hrs.	5 hrs.	5 days	7 days	5 days	2 days
B. anthracis (no spores) ...	0	0	2 days	3 days	3 days	2 days	2 days	0	4 days	5 days	3 days	2 days
V. cholerae	5 hrs.	5 hrs.	5 hrs.	5 hrs.	2 days	30 hrs.	30 hrs.	5 hrs.	5 hrs.	2 days	2 days	30 hrs.
B. prodigiosus	1 day	12 hrs.	2 days	5 days	15 days	2 days	8 days	12 hrs.	11 days	5 days	17 days	6 hrs.
Anthrax spores	10 days	4 days	4 days	8 days	7 days	5 days	20 days	20 days	20 days	13 days	20 days	13 hrs.

— = no experiment made. 0 = not usually cultivated.

General Summary and Conclusions.

Experiments on flies can be easily conducted in glass cages closed by gauze at one end, and standing on glass plates. In such cages the flies can be fed and kept alive for many days. Transference from cage to cage is easy, and flies for cultural purposes can be obtained without difficulty. Cultures should be made by inoculating the legs, wings and head and the contents of the crop and intestine separately on the surface of solid media, or into small tubes of fluid media. Flies feed readily on various fluids such as syrup, milk and sputum. The food first passes into the crop, and subsequently, if the meal is continued, directly into the intestine. If the meal is discontinued after the crop has been distended its contents gradually pass, during the next few hours, into the intestine. After a meal, flies usually regurgitate some of the contents of their crops through the proboscis. This fluid may either be sucked back again or deposited on the glass. For the sake of convenience the regurgitated fluid has been called "vomit." The proventriculus seems to act as a valve which is shut at the commencement of a meal in order to allow the food to pass first into the crop. Later it opens so that food may pass directly into the ventriculus from the œsophagus, or, if the meal has been discontinued, from the crop. It is probably closed when material is vomited. When feeding on semi-fluid or solid soluble materials, flies frequently moisten or dissolve them by means of vomit. On such substances as partially dried milk, imprints of proboscides are frequently seen. A fly which has access to abundant food produces between 15 and 30 deposits (vomits and fæces) in 24 hours.

Infection experiments show that non-spore bearing pathogenic bacteria do not usually survive more than a few hours (5–18) on the legs and wings. Nevertheless flies allowed to walk over sterile agar plates may cause infection for several days. This seems to be due to the fact that they frequently attempt to suck the surface, and in so doing infect it with fluid regurgitated from the crop. Within the crop non-spore bearing bacteria frequently survive for several days, and they usually survive even longer in the intestine. No evidence of multiplication in either of these situations has been obtained. The fæces deposited by such flies often contain the organisms in considerable numbers for at least two days, and are frequently infective for much longer periods. Anthrax spores survived for many days on the exterior and in the alimentary canal.

Experiments with *B. prodigiosus* show that flies may infect sugar 48 hours after feeding on infected material, and that clean flies may infect themselves by feeding on the recent deposits of infected flies. In the few experiments which were tried, milk and meat were not infected. Flies fed on anthrax spores did, however, infect the syrup which was given to them as food.

In the experiments which have been described, very gross infection was produced in most cases by emulsions of pure cultures. It is improbable, however, that under natural conditions flies would often have the opportunity of feeding on materials which contain pathogenic organisms practically in pure culture. The effects of contaminating with non-pathogenic and putrefactive bacteria have

as yet not been studied, and the effects of season, temperature, atmospheric conditions, different diets, irregular and scanty feeding, and other disturbing factors have not received sufficient attention. Consequently it would be premature to conclude that the experiments and observations described in this paper do more than indicate that, under exceptionally favourable conditions, certain bacteria can be recovered from the contents of the alimentary canal and fæcal deposits of infected flies for several days after infection; and that these flies are capable of infecting certain materials on which they feed for several days. The experiments with tubercular sputum and anthracic blood alone afford evidence as to the duration of life in the contents of the alimentary canal of pathogenic bacteria taken up under natural conditions.

That flies sometimes do become grossly infected under natural conditions is shown by the fact that in a few instances pathogenic bacteria have been isolated from naturally infected flies. Simmonds (1892) isolated cholera vibrios from flies which were captured in a post-mortem room in which the bodies of persons dead of cholera were lying. Tsuzuki (1904) was able to cultivate the same organism from flies captured in a cholera house, and Tizzoni and Cattani (1886) obtained cultures from flies caught in cholera wards. Hamilton (11. 1903) and Ficker (1902) isolated *B. typhosus* from flies caught in houses in which persons were lying ill of typhoid fever, and Faichnie (1909) obtained *B. typhosus* from a number of flies caught in various places where typhoid fever prevailed. He further showed that *B. typhosus* or *B. paratyphosus* (A) could be cultivated for several days from the intestines of perfect insects which emerged from larvæ fed on fæces containing these organisms.

Several observers (Celli (1888), Hayward (1904), Lord (1904) and Buchanan (1907)) have shown that the fæces of flies which have fed on tubercular sputa contain virulent tubercle bacilli. Buchanan (1907) demonstrated that flies which had walked over naturally infected anthracic meat were capable of infecting agar plates. Yersin (1894) in Hong-Kong observed many dead flies lying about in his laboratory where he made autopsies on plague animals. He demonstrated by inoculation into animals that a dead fly contained virulent plague bacilli.

Finally the experiments of Macrae (1894) at the Gaja jail show that exposed milk may become infected by the agency of flies.

Even these observations only prove that cultures of pathogenic organisms may occasionally be obtained from naturally infected flies, and they do not afford conclusive evidence that such flies are a frequent source of disease in man by infecting food materials. Though many of the observations cited by Nuttall and Jepson seem to indicate that flies have frequently acted as carriers of disease, it has only once (Macrae) been demonstrated that food has actually been grossly contaminated by them.

The writer hopes to continue these experiments with naturally infected materials both with *M. domestica* and other non-biting flies.

G. S. Graham-Smith.

PLATE I.

FIG. 1.—Glass cage in which infected flies were kept, consisting of a glass cylinder 9 × 3 inches, covered with gauze at one end and open at the other. ($\frac{1}{6}$ nat. size.)

FIG. 2.—Glass cage with apparatus, through which to extract flies, in place. The latter consists of a board in which a round hole slightly larger than the diameter of the cage had been cut and lined with cloth so as to grip the sides of the cage. On to the cloth gauze was sewn to form a conical bag open at the free end. ($\frac{1}{6}$ nat. size.)

FIG. 3.—Gauze bag in which normal stock flies were kept. Note the sleeve at the side through which the flies were extracted. ($\frac{1}{6}$ nat. size.)

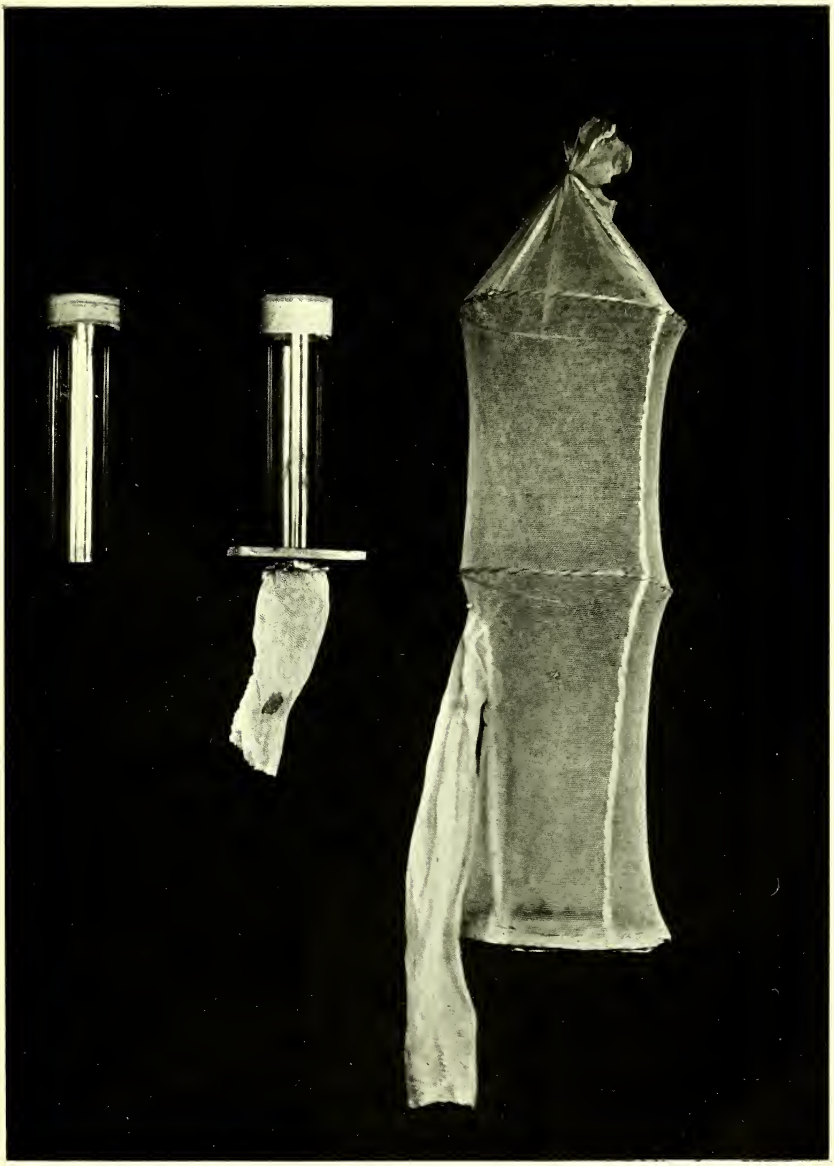

Fig. 1. Fig. 2. Fig. 3.

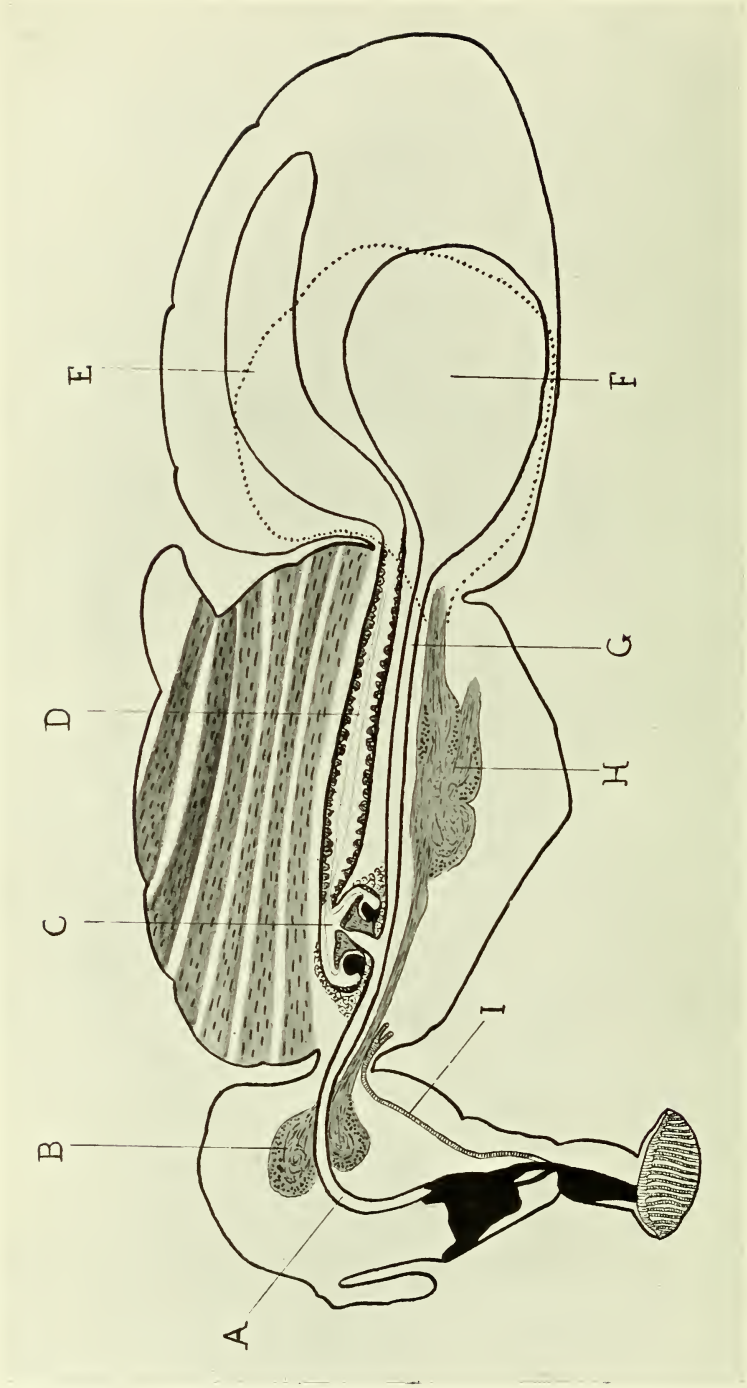

Fig. 4.

PLATE II.

FIG. 4.—Schematic longitudinal section of a fly. Oesophagus A, Cephalic ganglion B, with oesophagus passing through it, Proventriculus C, Ventriculus D, Proximal intestine in abdomen E, Crop F, Crop duct G, Thoracic ganglion H, and Common salivary duct I.

The dotted line indicates the position of one of the large abdominal air sacs. Compare with Plate III, fig. 5.

PLATE III.

FIG. 5.—Photograph of a longitudinal section of a fly (× 18). 1, oesophagus passing through the cephalic ganglion ; 2, the oesophagus entering the proventriculus ; 3, the proventriculus ; 4, the ventriculus ; and 5, the crop duct.

FIG. 6.—Photograph of a longitudinal section of the head and anterior portion of the thorax (× 20). 1, pharynx ; 2, oesophagus, showing its passage through the cephalic ganglion, and the bend after it emerges from the pharynx ; 3, oesophagus entering the proventriculus ; 4, proventriculus ; 5, common salivary duct.

(The dorsal region of the oesophagus in the neck has been accidentally ruptured.)

FIG. 7.—Photograph showing the proventriculus in longitudinal section (× 50).

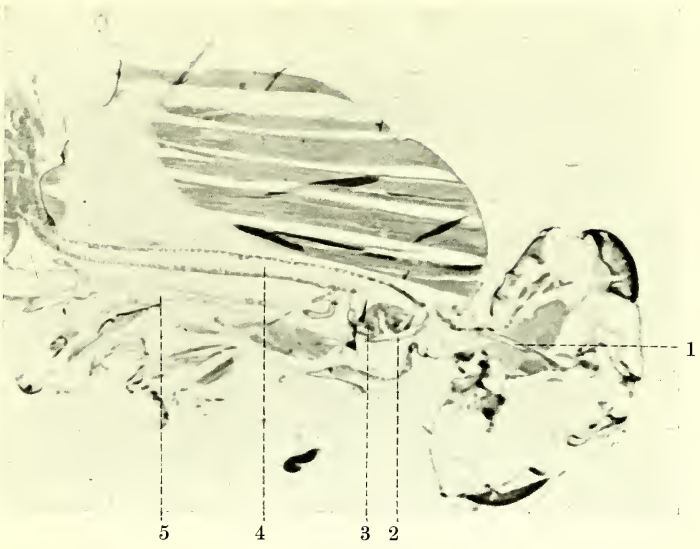

Fig. 5.

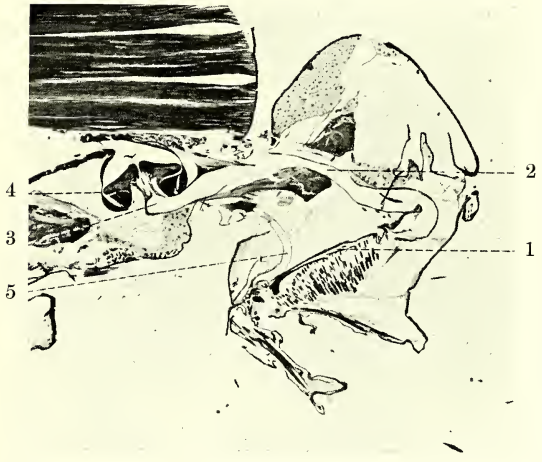

Fig. 6.

Fig. 7.

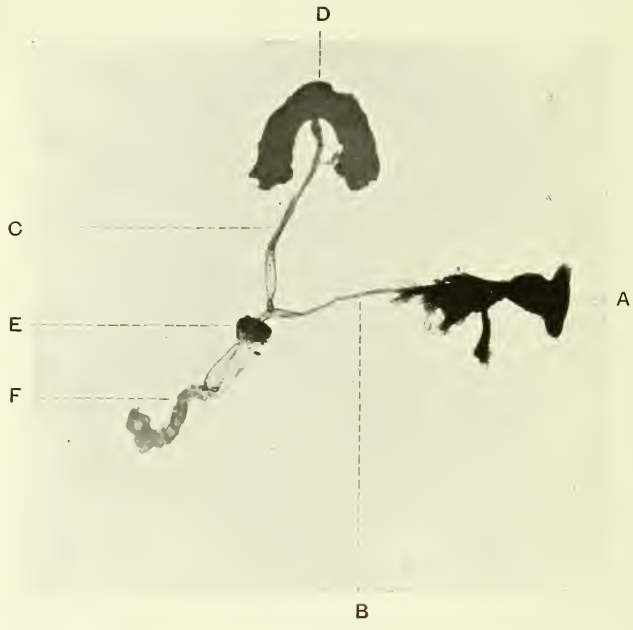

Fig. 8.

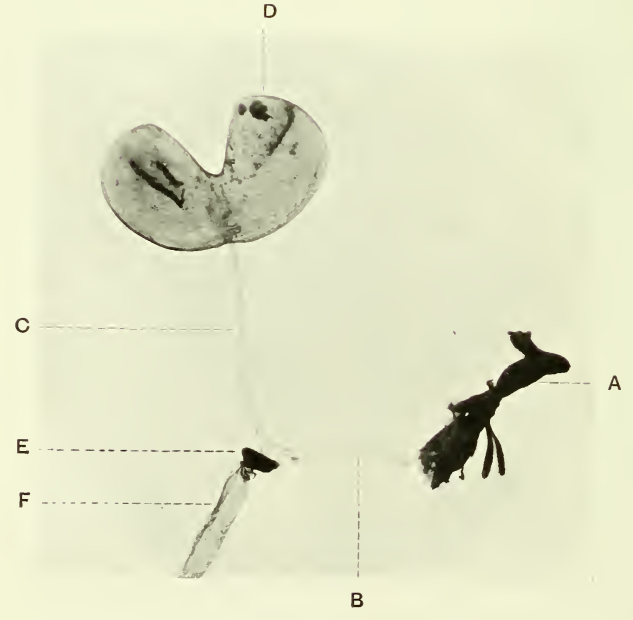

Fig. 9.

PLATE IV.

FIG. 8.—Photograph of a dissection (× 16) showing the proboscis (A), oesophagus (B), crop duct (C), crop, almost empty (D), proventriculus (E), and ventriculus (F) of a hungry fly.

FIG. 9.—Photograph of a dissection showing the same structures in a fly recently fed on gelatin. Note distension of the crop.

PLATE V.

FIG. 10.—Photograph (side view) of an unfed fly (× 7).

FIG. 11.—Photograph (side view) of a fly shortly after feeding on syrup. The distension of the anterior ventral portion of the abdomen in which the crop lies is well seen.

FIG. 12.—Photograph (× 7) of the ventral surface of a fly recently fed on syrup, coloured with carmine. The dark area in the anterior portion of the abdomen, which was coloured red, indicates the position of the crop.

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

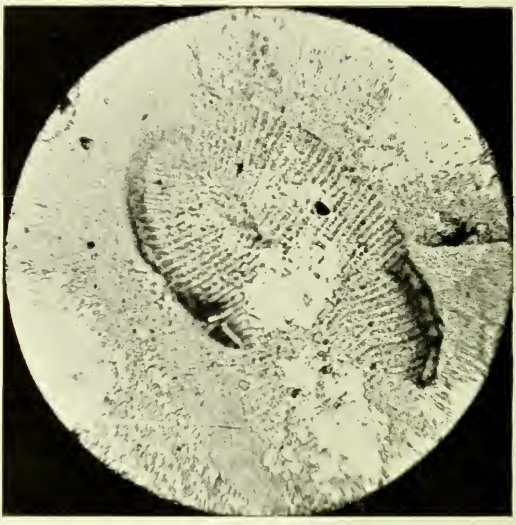

Fig. 14.

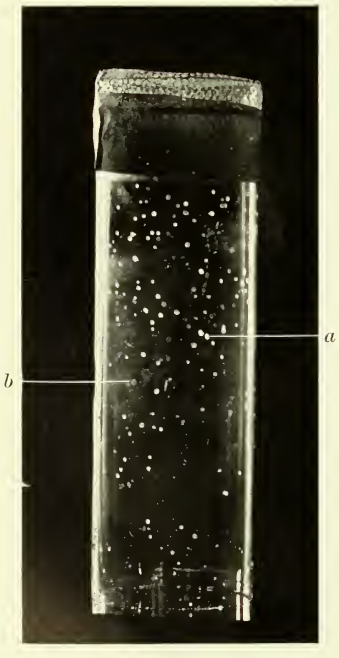

Fig. 15.

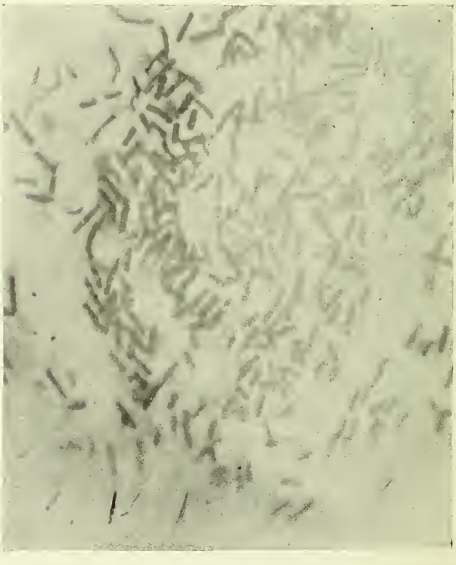

Fig. 16.

PLATE VI.

FIG. 13.—Photograph ($\frac{3}{4}$ nat. size) of three rounded patches of milk smeared on glass and allowed to partially dry. The round or oval markings seen all over these patches were made by the proboscides of flies allowed to feed on them.

FIG. 14.—Photograph ($\times$ 50) of a "proboscis mark" on one of the patches illustrated in Fig. 13, showing the contour of the oral lobes and the imprints of the pseudotracheae.

FIG. 15.—Photograph of a cage ($\frac{1}{2}$ nat. size) in which well fed flies had been kept. Its surface is covered by numerous "spots." The white ones (*a*) are faecal deposits and the lighter ones (*b*) with dark centres "vomit" marks.

FIG. 16.—Photograph of a film preparation made from the crop contents of a fly (Table 17, No. 11) three days after feeding on the blood of a mouse just dead of anthrax. The preparation consists of a nearly pure culture of non-spore bearing anthrax bacilli.

PLATE VII.

FIG. 17.—Photograph ($\frac{2}{3}$ nat. size) of an agar plate before incubation, inoculated with the organs of four flies infected with *B. anthracis*. The cultures from each fly are separated from each other by lines drawn on the bottom of the plate, and are numbered I, II, III, IV. In each case the parts inoculated with the crop and gut contents and the fluid expressed from the proboscis are surrounded by circles and marked C, G and P respectively. The legs, wings and heads have been separately inoculated in each case.

FIG. 18.—Photograph of the same plate after 24 hours' incubation at 37° C. Large anthrax colonies have developed in the places inoculated with the crop and gut contents of fly III. Colonies of *B. anthracis* and other organisms have grown round several of the other inoculated portions of the plate.

FIG. 19.—Photograph of a plate culture made from the legs, head and contents of the abdomen of a fly, which died of infection with *E. muscæ* 14 days after feeding on syrup infected with the spores of *B. anthracis*. The body was subsequently kept in a glass bottle and the culture made 155 days after death. Colonies of *B. anthracis* have developed round the legs and several portions of the abdominal contents.

Fig. 17.

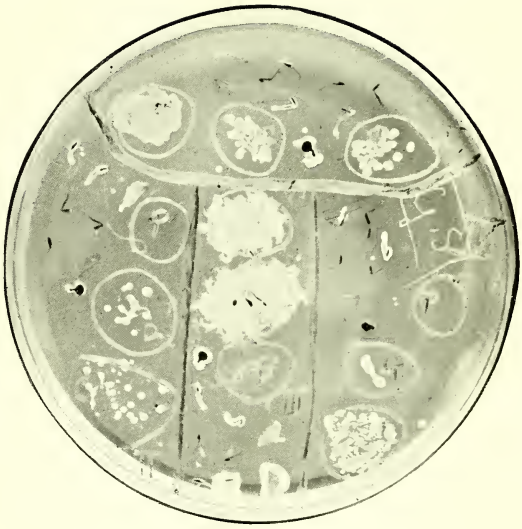

Fig. 18.

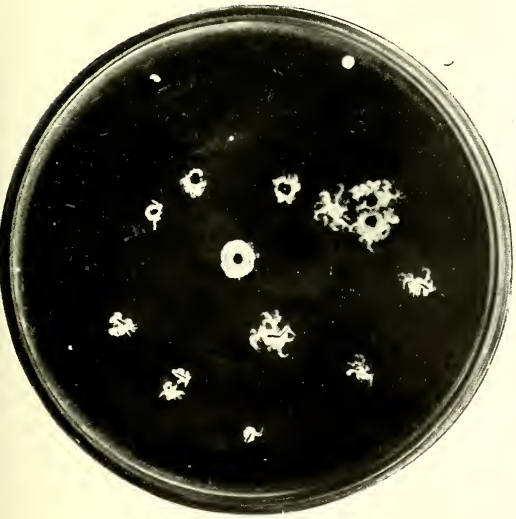

Fig. 19.

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Summary of Literature Relating to the Bionomics of the Parasitic Fungus of Flies; *Empusa Muscæ*, (Cohn), with Special Reference to the Economic Aspect: by Julius Bernstein, M.B., M.R.C.P., Bacteriologist, City of Westminster.

The most effective natural enemy of the house fly is the fungus disease *Empusa muscæ*. It is specially prevalent in the months of July, August and September, causing the death of the flies in large numbers, more especially towards the end of this period; but though large numbers are destroyed by it the excessive rapidity of development at the beginning of the following season may soon more than replace those destroyed. If, however, nature could be assisted in her methods, and the fungus brought into contact with the flies, or their larvæ, by scientific methods, it seems probable that the number of flies might thereby be brought under better control. To this purpose a knowledge of the complete cycle of development, methods of infection, and the conditions which determine the persistence of vitality of the fungus from the end of one fly season to the beginning of the next is necessary. Knowledge on these subjects is, however, not yet complete. In analogous cases of infection, however, work has been done which will perhaps be of assistance in this enquiry.

It is known that the spores of this fungus can attach themselves to the body of a fly and vegetate into hyphæ. These penetrate into the body cavity and form gemmæ which are disseminated throughout the body, and in a few days germinate into hyphæ which ramify in all directions (Brefeld). The fly in a short time succumbs to the parasitic invasion, and is found on the windows and walls dead, but in a life-like attitude. A few hours after death the fungus has further germinated and appears on the surface of the body forming a mycelial net-work from which conidiophores project, from the terminals of which spores are projected to a distance of about one inch, forming within this radius a whitish zone around the fly. (Varley and Cohn).

Like other varieties of Entomophthoræ the *Empusa muscæ* is peculiar to its host the house-fly (*M. domestica*). But, strangely, though its hosts are frequently found out of doors the fungus is only very exceptionally so found. (Thaxter.)

The conidia live only a few days (Olive), but can spread some distance by germinating into conidiophores which give rise to spores. These again are capable of forming secondary conidiophores which, in turn, may also germinate. (Hewitt.)

Mode of Infection.

There is much yet to be learned under this heading. Confinement of healthy flies with those that have died from the disease does not necessarily result in infection (White), though similar experiments with caterpillars and *Empusa Grylli* (an allied fungal parasite) has resulted, under special conditions, in producing infection. Brefeld successfully inoculated the spores of *E. muscæ* through the thin white parts of the skin on the under surface of *M. domestica*, and obtained germination of the conidia on the surface of the fly. Locusts have also been directly infected with *E. Grylli*, by spraying with water in which spores had been suspended. (Pole Evans.) Olive, working recently with *E. Sciara* on flies cultivated on horse-dung in a laboratory, could not definitely determine the manner of infection. His preliminary experiments on infecting healthy adult larvæ suggested that infection occurred in the very young, whilst on the surface of the dung, and before they burrowed into the depths to mature. He also was unable to determine whether the disease could be carried from the larvæ to the adult fly.

Persistence of the Fungus from Season to Season.

This question has not yet been fully worked out. The conidia which are formed under certain atmospheric conditions—moisture &c.—have been shown to be not very viable, so can hardly be the means of carrying the species over from the end of one season to the beginning of the next. It would, of course, seem probable that resting spores are formed, but though these have been described (Winter and Giard), the evidence as to their existence is not very definite. It has also been suggested that the species is kept alive in those few house-flies which have been shown to develop in stables, bake-houses, &c., under favourable conditions of food and and temperature in winter. (Gordon Hewitt and Jepson.)

The Fungus as an Economic Factor.

It was only natural that attention should be directed to the utilization of the Entomogenous fungi for economic purposes, and as far back as 1858 Professor Sebert made the suggestion. (Massee.) Since then much has been done on these lines in France against the larvæ of the common cockchafer, in South Africa and India against the locust with *E Grylli*, and in the United States against the chinch-bug with *E. Aphidis* ; but the results have been most unsatisfactory. The difficulties to be encountered are the artificial cultivation of the fungus and the inoculation of the insects under suitable conditions, among which climatic conditions as to moisture &c., are of the utmost importance.

All attempts to cultivate the *Empusæ* on artificial media have practically resulted in failure. Sheldon, in 1903, attempted to grow the spores of *E. Grylli* that were shed on to the glass Petri dishes in which dead grasshoppers and caterpillars were kept under

certain conditions of moisture, but only obtained one successful result out of two hundred experiments : he used, as a culture medium, agar, and intended experimenting further with glycerine-agar, but apparently no record of any further experiments by him has been published. A fungus, cultivated in large amounts in Grahamstown, South Africa, and widely distributed for many years as *E. Grylli*, was used extensively but ineffectually in the campaign against locusts. This, however, was eventually shown to be not the true parasite but a harmless saprophyte—*Mucor Exitiosus*, Massee (l. c.).

Cohn, as early as 1885, mentioned an overgrowth of saprophytes on the bodies of the flies killed by *Empusæ*, and recent observers have experienced great difficulty in isolating any particular fungus, such as *Empusa*, *Botrytis*, *Sporotrichum*, *&c.*, owing to the fact that these are almost invariably accompanied by saprophytic fungi such as *Mucor*, *Aspergillus*, *&c.*, which, under conditions of experimental cultivation, quickly crowd the others out. It may, of course, eventually prove possible to obtain suitable media on which an artificial cultivation of individual species may be grown. Analogous conditions would indeed encourage this hope : for example, the special methods which have proved successful in the case of *Trichophyton*.

Then arises the question of artificial infection of the insects. Nothing appears to have been done as yet in the case of *M. domestica*, but Pole Evans (l. c.) successfully infected locusts, as mentioned above, and Webster was able to spread disease amongst the chinch-bugs, in hills of corn, by using the fungus present in the powdered bodies of bugs which had died from the disease.

Both observers are far from hopeful of results on a sufficiently large scale, and Webster points out that the fungus can only be of practical use in agriculture in cases of excessive abundance and massing together of the bugs in wet weather, and provided there is an abundant and readily accessible supply of the fungus at hand. Other American and Indian authorities are equally pessimistic (Bruner) ; but Dwight Sanderson remarks : "So far we must confess to rather poor results with fungi and bacteria used as artificial means for insect control, but they are undoubtedly large factors in the control of nature."

In the case of *M. domestica*, perhaps the question may be a little more hopeful. The breeding places, haunts, and habits are well known. Flies do not travel over such long distances as the locusts, and may, perhaps, prove to be more easily attacked than the chinch bugs, for instance, on account of their close association with household life. Moreover, it has been shown in the case of *E. Sciara*, that the larvæ themselves can be infected, and consequently this may also be found to hold as regards *E. muscæ*.

Conclusions.

The house-fly *M. domestica*, in common with many other insects, is prone to the attacks of a fungus which is practically peculiar to it, and which slays it in great numbers. The fungus itself soon dies out, and it is not at present known how it persists from the end of one season to the commencement of the next. It has never been grown in artificial cultures, and there is much yet to be learned

about its mode of infecting its specific host. In economic entomology, experiments on a large scale have been unsuccessful, but this must be discounted in these particular experiments by the fact that for a series of years saprophytic contaminations were mistaken for, and used in place of, the specific parasitic fungi. It is evident that before any attack can be made on the house-fly by concentration of nature's method of infection, more must be known about the life history of the *E. muscæ* and its method of infection. There does not seem to be any reason why suitable artificial media on which to grow the fungi should not be found, and experimental work, having this special object in view, has already been commenced. If, as a result, the fungus should prove to be capable of artificial cultivation in sufficiently large amounts, further investigation will be made as to the probable value of its employment on a large scale.

I have to thank Mr. Massee, of Kew Gardens, for much valuable assistance in directing me to the literature of the subject, and Mr. Austen, of the British Museum (Natural History), for similar kind assistance.

JULIUS BERNSTEIN.

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J.B.

Note as to work in hand, but not yet published; and as to proposed further work in reference to Flies as Carriers of Infection: by S. Monckton Copeman, M.D., F.R.S.

As having, obviously, an important bearing on possible carriage of infection, it is proposed during the present year to devote special attention to the elucidation of the question as to the range of flight of flies, both in horizontal and vertical directions; and under varying conditions, meteorological and otherwise. The results of experiments as to the suitability of various methods of colouring flies, for purposes of identification, carried out by Mr. Jepson, in 1909, and

published in the second of this series of reports (pp. 4–9) have indicated the possibility of marking large numbers of flies with a minimum of trouble, and yet so efficiently that, under experimental conditions, at any rate, they can be identified with certainty for as long a period as 20 days.

Arrangements have been completed, at Cambridge, for the location of a number of stations at which fly-traps of various kinds will be installed. These stations have been arranged, radially, around a central experimental station, from which, at the first favourable opportunity, it is proposed to loose large numbers of specially marked flies. The first circle of traps will be set quite near to the central station, while the remaining traps will be set in circles, the radius of which will in each case increase by approximately 50 yards, up to a maximum radius of 400 yards. The relative positions of the various fly-stations, at which arrangements have been made for traps to be set, is shewn on the accompanying outline plan reduced from the 25-inch ordnance map of Cambridge.

The flies trapped at these different stations will be examined and counted with special reference to " marked " individuals, at frequent intervals—daily, if possible - and the results entered on specially-prepared charts. Control specimens of the marked flies will be kept at the central station, in order to have an approximate means of judging as to how long the experimental colouring of each batch of flies set free is likely to be recognizable.

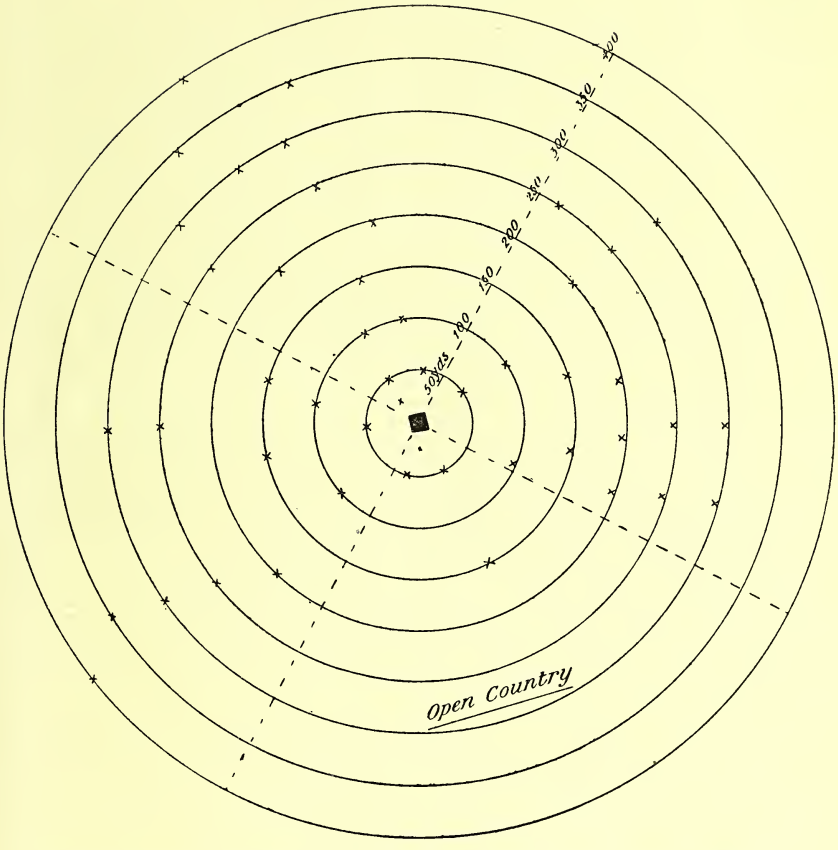
It is also intended, if possible, to carry out, simultaneously, a similar series of experiments around a new experimental farm which is being opened on the out-skirts of Cambridge. This locality, which is but sparsely populated and built over, will afford, as regards experimental conditions, a useful contrast to those obtaining in the town itself. During the progress of these experiments full and exact records of meteorological conditions will be kept. The conduct of these experiments is entrusted to Mr. Gordon Merriman, under the general direction of Professor Nuttall.

Trial will also be made, at Cambridge and elsewhere, of the respective value of the various baits that have been proposed from time to time for attracting and killing flies. Incidentally the breeding places most favoured by *Homalomyia Canicularis* (the lesser house-fly), with special reference to the nature of the material in which, by preference, oviposition takes place, will also form subject of investigation.

Dr. Graham-Smith will continue his work on the experimental infection of flies with pathogenetic micro-organisms. He also proposes to carry out further observations on the anatomy and function of the proventriculus of the fly in connection with the regurgitation of infected material ; and to initiate a series of breeding experiments dealing with the question of possible infection during the larval stages.

To Dr. Nicoll, of the Lister Institute, has been entrusted an inquiry into the possible agency of flies as carriers of infective organisms other than bacteria, more particularly of the ova of parasitic worms.

In continuance of the paper published in the present report, Dr. Bernstein will undertake a detailed investigation of the life



CAMBRIDGE INVESTIGATIONS ON THE RANGE OF FLIGHT OF FLIES.

Plan showing approximate situation of the Stations (×) at which fly-traps and fly-papers are to be located.

■ = Central experimental station from which "marked" flies will be set loose.

history of *Empusa muscæ* the parasitic fungus of the fly, and will attempt its cultivation in artificial media, with the object, if possible, of employing such artificial cultures for the destruction of flies on a large scale.

From Dr. Laver, of Colchester, I have received interesting information, not as yet, however, sufficiently detailed for publication, bearing on the debateable question as to the stage in their life history in which flies are capable of surviving from the end of one fly season to the commencement of the next.

The evidence at present available on this point appears to indicate that, in addition to the more or less active individuals which are usually to be found, throughout the winter months, in bakehouses and other warm situations, considerable numbers of flies in the adult stage, hybernate outside dwellings in various sheltered situations, the under surface of the thatch of farm-yard stacks having been found by Dr. Laver to constitute specially favoured winter quarters.

Arrangements which had been made for study, during the past two years, of the relationship between the curves representing prevalence of flies, and incidence of epidemic enteritis (summer diarrhœa) and of enteric fever respectively, have not been productive of useful results, owing in large measure to the somewhat abnormal meteorological conditions (low average temperature and persistent rainfall) that have been experienced during the period under consideration. This question has, however, received exhaustive consideration by Dr. Niven, Medical Officer of Health of Manchester, in a paper presented* to the Epidemiological Section of the Royal Society of Medicine, in which he presents, and critically discusses, the records of investigations that have been carried out, under his direction, at Manchester, over a considerable number of years.

Reference should also be made to recent special reports of the Medical Officer to the London County Council,† in which Dr. Hamer records and discusses the results of investigations, on somewhat similar lines, which he has conducted in London during the past three years.

It may be mentioned that Dr. Niven and Dr. Hamer are in general agreement as to the facts, although they differ somewhat as to the interpretation to be placed upon them. Circumstances permitting, it is hoped that the present year will give opportunity for work of similar nature to be carried out in a number of large towns. The differing sanitary circumstances of these viewed in connection with the results obtained in each instance, may be expected to afford more accurate knowledge than is at present available on the precise relationship of fly prevalence to the incidence of certain intestinal diseases in epidemic form.

S. Monckton Copeman.

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*Summer Diarrhœa and Enteric Fever by James Niven, M.B. *Proceedings of Royal Society of Medicine*, Vol. III., No. 6, April, 1910.

† Nuisance from Flies : Reports to London County Council by the Medical Officer of Health presenting reports by Dr. Hamer on the extent of which fly nuisance is produced in London by accumulations of offensive matters. Annual Report of Public Health Committee, London County Council, 1907 and Special Report No. 1207, 1908.